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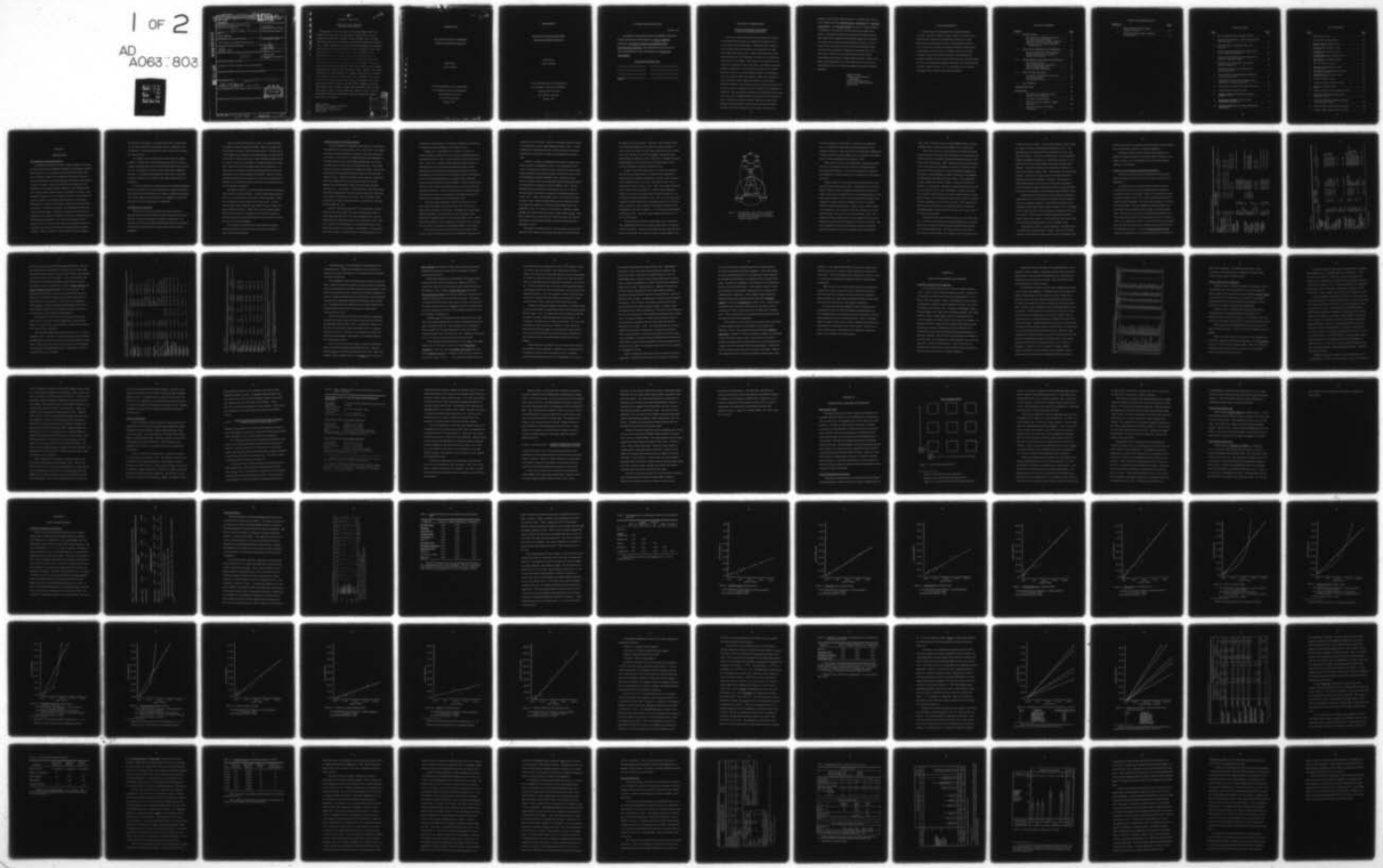

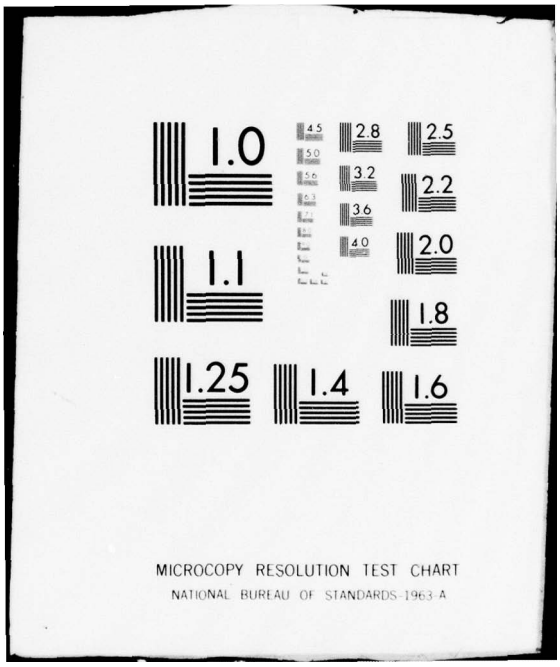
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ABSTRACT OF DISSERTATION

SELENIUM IN AQUATIC ORGANISMS
FROM SELENIFEROUS HABITATS

The purpose of this study was to locate inland water habitats in Colorado and Wyoming that are influenced by selenium (Se) to varying degrees and compare them. Samples of water, sediment, flora, and fauna from these habitats were analyzed for Se using atomic absorption spectroscopy. Aquaria experiments with native fish using lake water and indigenous food sources were employed to study the route of Se uptake. Thirty sites were surveyed and mean levels up to 15.8 µg Se/l water were found. Correlation-regression analysis of organism Se level data from 18 of these sites revealed that those levels can be reliably predicted based on a knowledge of water and sediment levels of the element. The experiments with native fish revealed that their tissue levels were more strongly influenced by the level of Se in food than in water. Because of the strong correlations that were measured, it is apparent that several of the organisms studied can serve as reliable quantitative indicators of the extent to which Se has entered aquatic food chains. In organisms from the more seleniferous habitats studied, whole body levels in apparently thriving fish ranged up to 60 µg Se/g dry weight and exceeded 100 µg Se/g in fish liver tissue. Levels in invertebrates were similar to those in fish but multicellular algae and mixed-species planktonic samples were somewhat lower (up to 40 µg Se/g).

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Department of Zoology and Entomology
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Spring, 1978

DISSERTATION

SELENIUM IN AQUATIC ORGANISMS FROM SELENIFEROUS HABITATS

Submitted by

John H. Birkner

In partial fulfillment of the requirements

for the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY JOHN H. BIRKNER ENTITLED SELENIUM IN AQUATIC ORGANISMS FROM SELENIFEROUS HABITATS BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

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Adviser

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SELENIUM IN AQUATIC ORGANISMS FROM SELENIFEROUS HABITATS

The purpose of this study was to locate inland water habitats in Colorado and Wyoming that are influenced by selenium (Se) to varying degrees and compare them. Samples of water, sediment, flora, and fauna from these habitats were analyzed for Se using atomic absorption spectroscopy. Aquaria experiments with native fish using lake water and indigenous food sources were employed to study the route of Se uptake. Thirty sites were surveyed and mean levels up to 15.8 μg Se/l water were found. Correlation-regression analysis of organism Se level data from 18 of these sites revealed that those levels can be reliably predicted based on a knowledge of water and sediment levels of the element. Other water parameters such as dissolved sulfate (chemically similar to water soluble selenate), hardness, and conductivity were not found to correlate with water Se levels or to influence the level to which it is accumulated in organisms. The experiments with native fish revealed that their tissue levels were more strongly influenced by the level of Se in food than in water. This agrees with the results of radiotracer studies which indicated that the principal route of Se entry into the food chain is probably via producer organisms such as algae. Because of the

strong correlations that were measured, it is apparent that several of the organisms studied (Hyalella azteca, Chironomus Sp., Astacidae, Cenocorixa Sp., and Fundulus kansae) can serve as reliable quantitative indicators of the extent to which Se has entered aquatic food chains. In organisms from the more seleniferous habitats studied, whole body levels in apparently thriving fish ranged up to 60 μg Se/g dry weight and exceeded 100 μg Se/g in fish liver tissue. Levels in invertebrates were similar to those in fish but multicellular algae and mixed-species planktonic samples were somewhat lower (up to 40 μg Se/g). These levels are considerably in excess of those recommended as being safe in animal feed (4 μg Se/g). However, the public health implications are unclear because of the uncertainties regarding the toxicity of Se when incorporated into tissues of aquatic organisms.

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INTRODUCTION

The Importance of Selenium Research

Information about selenium levels in aquatic habitats is an essential part of the general developing knowledge of environmental selenium. Selenium (Se) is one of a special class of elements that have a vital role in biological systems even though they are present at very low (trace) levels relative to the more abundant constituents of life such as calcium or nitrogen. Research about these trace elements can be undertaken in any context from the subcellular to the biosphere level. This is because these elements must, to one degree or another, cycle through the organism and its environment if they are to be available in amounts conducive to the growth and reproduction of living things. Deficiencies or excesses in availability, whether due to natural local variations or to interference by man, are reflected by the quality and quantity of living things. Fertilizers for plants and dietary mineral supplements for animals are examples of efforts to remedy deficiencies. Contamination and pollution are in some cases examples of trace element excess. In the case of Se, there are recognized instances of both deficiency and excess with deficiency being the more prevalent condition. While it is difficult to say to what extent these problems

will increase in the future, it is likely that the more we learn about Se, the more concerned we will become about its availability in the environment. The questions pursued in this research are in anticipation of this concern.

Se is known to be an essential trace level nutrient for higher animals. Although it has not been demonstrated, there is reason to suspect that appropriate levels of environmental Se may also be necessary for the metabolic function of at least some aquatic organisms. Moreover, it is possible that appropriate levels of Se in the tissues of aquatic organisms are of benefit to the higher animals which consume them.

Se is also regarded as a potentially toxic environmental pollutant. Excessive availability of Se has the potential for causing tissue levels that would not only be detrimental to the aquatic organisms themselves but to others higher in the food web as well. Appendix 1 outlines the chemistry of Se and its importance in metabolism.

The Objectives of This Study

This study concerns environmental and tissue Se levels in selected lake and reservoir habitats located in the plains and foothills adjacent to the Rocky Mountains of Colorado and Wyoming. This region is one of widely varying Se availability which in turn influences environmental concentrations and levels of the element in aquatic organisms.

Several related questions that are basic to an understanding of Se dynamics in aquatic habitats are posed. What is the range of Se levels found in the water from the various habitats? Do the Se levels in the organisms and sediments from these habitats correlate with the water values? Does the presence of other dissolved substances such as sulfate or those which contribute to hardness influence the correlation? What is the route of Se uptake from water to organisms? To what extent is it possible to predict the consequences for fresh water organisms of man-caused alteration of Se levels? Answers to these questions will help form the foundation for future work regardless of whether it is involved with theoretical concerns or the practical problems of deficiency and excess.

The approach taken here will be different than that used by many others who have studied Se effects. In those studies (discussed later) Se in various forms has often been added to aquatic systems. Radioactive isotopes have been used to study turnover rates. Various purified reagent forms of Se have been added to water or food in bioassay studies of Se toxicity. Rather than contrive conditions under which to study Se effects, the approach of the present research is directed toward the study of habitats which are the influence of natural sources of Se to varying degrees.

The introductory sections which follow, outline the relevant information which has been previously reported concerning Se in aquatic habitats and organisms.

Selenium Availability in Aquatic Habitats

Se ranks seventieth in abundance in the Earth's crust having an average level of 0.05 μg/g (Crystal, 1973; Lakin, 1973). The expression μg/g (micrograms/gram) is equivalent to the term, ppm (parts per million). As is the case in biological systems (see Appendix 1), Se tends to mimic sulfur in its geochemical dynamics and is found with that element in many sulfur-containing minerals. The average ratio of occurrence is one Se atom to 6000 sulfur atoms. The environmental aspect of Se that has been most studied is its presence and mobility in soils. While all rock types contain some Se, weathering shales are considered one of the principle sources for soil Se. Igneous rock and sedimentary forms other than shale contribute relatively less. For example, in North America, those shales identified with the inland seas of the late cretaceous period (70-100 million years before the present) are rich in Se. This is believed to have been partly the result of extensive volcanic release of Se-containing material (Oldfield, 1974).

Minerals containing selenide (Se^{-2}), elemental (Se^{o}), selenite (SeO_3^{-2}), and selenate (SeO_4^{-2}) forms of Se all may be present in weathering parent material. The relative proportions are not well understood but certainly vary from area to area (Olsen, 1967). However, selenate is the most likely to be transported by water in dissolved form because of its solubility. See Appendix 1 for discussion of Se valence states. Selenide minerals and elemental Se are very

insoluble and selenite tends to be rendered insoluble by the formation of compounds with iron (Oldfield, 1974; Howard, 1971).

Climate has a substantial effect on whether or not Se will remain available in soluble form once it has been weathered into soil from parent material. Moist, acidic soils tend to lose mobile forms of Se. This is due to chemical and possibly microbial reduction to insoluble forms such as selenite and elemental Se which remain immobilized and unavailable for leaching and absorption by plants. See Appendix 2 for a discussion of microbial selenium transformation. In arid or semiarid alkaline areas, Se supplied from weathering parent material will tend to be more available. Aerated, alkaline soils provide a more oxidative environment which over time will favor the formation of selenate. This form of the element is available to plants and is also more subject to leaching when sufficient precipitation or ground water is supplied (Lakin and Davidson, 1966).

It has been shown that plants may have an important role in inter-converting various forms of Se (Olsen, 1967) which in turn affects soil availability of the element. Investigations of plant uptake (Hamilton and Beath, 1963) show that the Se accumulator plants described in Appendix 4 produce water-soluble organic forms. When the plants decompose, these compounds are returned to the soil where they are subject to transport by leaching and uptake by other plants

The actual soil levels of Se which have been measured range from 0.1 μg/g in Se deficient areas of New Zealand to 1200 μg/g in an

organic-rich area in Ireland. However, knowledge of total Se content in a given soil is not very useful information (Lakin, 1973). It is the mobility of Se compounds which has the most bearing on how plants, ground water, and surface drainage will be influenced by the Se in soils.

Airborne transport and deposition of aerosols is another factor which can affect Se availability in water. Se is mobilized into the air as a consequence of volcanic activity, ocean and terrestrial water spray evaporation, wind borne soil dust, and volitization from soil by microorganisms and plants (Oldfield, 1974). Animals are also a source of volatile forms such as dimethyl selenide $[(CH_3)_2 Se]$ which they liberate from respiratory surfaces (Allaway, 1973). The predominant source of industrial Se emission into the air is from the burning of fossil fuels and the refining of Se-containing sulfide ores (Lakin, 1973). Unlike sulfur dioxide, which can be emitted as vapor, Se oxides such as selenium dioxide (SeO_2) are particulate and would not be expected to disperse as widely as sulfur dioxide (Frost and Ingvolstad, 1975). Se is used in the electronics, metallurgic, glass, pigment, and rubber industries as well as others (Cooper et al., 1974). Some emission into air and water will result from these and from the incineration of the products when they are discarded (Subcommittee on Selenium, 1976).

The relative contributions to air from the various sources is not known and there seems to be no information about how much airborne

Se might find its way into water. Moreover, only a limited number of reliable determinations of Se levels in air have been made. A recent review concluded that the average concentration is well below 0.01 $\mu g/m^3$ and that most of this is probably in particulate form (Subcommittee on Selenium, 1975). This level is 1/2000th the recommended maximum of 0.2 mg/m^3 set by the United States for airborne Se compounds in industrial workroom areas.

In spite of the limited information, it is clear from existing data that Se is an element which is mobilized and transported in several forms by numerous mechanisms. For this reason, mention of the "selenium cycle" is in order. One version of this cycle is reproduced in Figure 1 (Olsen, 1967). Only a few attempts have been made to quantify the flows depicted. The flow from eroding land masses to the oceans via flowing water has been estimated at 7.2×10^9 g/year (Bertine and Goldberg, 1971). For air, one recent report estimated worldwide emissions to be approximately 8.2×10^8 g/year (Andren and Klein, 1975). Another report gave an estimate for the United States alone that was higher - 1.1×10^{10} g/year (Subcommittee on Selenium, 1976). The same report estimated solid waste Se to be 3.1×10^{10} g/year.

With regard to the subject of solid waste, some investigators have considered the fate of Se in sewage sludge when it is reclaimed for use as fertilizer. Because of the highly reducing conditions which exist in this material, one authority assumes sewage sludge Se will

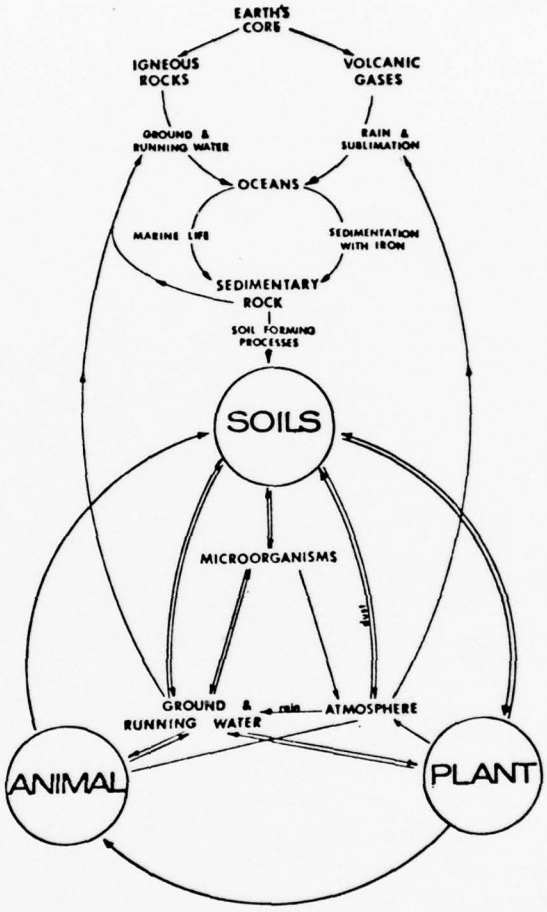

Figure 1. The selenium cycle. The environmental compartments and avenues of exchange believed to be important to the cycling of selenium (Olsen, 1967).

become insoluble and immobilized. In this form, Se would tend not to be available for plant uptake or leaching (Allaway, 1973). Inorganic fertilizers, unless they have been intentionally supplemented, are also considered to be an unlikely source of significant Se addition to soil or water (Subcommittee on Selenium, 1976).

While the Se cycle is not well understood, the pathways shown do serve to summarize the various avenues of exchange between environmental components. The water in which aquatic organisms find themselves is clearly influenced by a complex array of Se inputs and losses.

Selenate (SeO_4^{-2}) is the most likely dissolved form that contributes to the levels measured in water. As indicated earlier, selenite (SeO_3^{-2}) tends to remain complexed in insoluble form and other inorganic forms such as elemental Se are very insoluble (Howard, 1971). Many organic forms of Se such as methyl selenide $[(CH_3)_2Se]$ from biological reduction could also contribute as well as very finely divided Se-containing particulates. Organic forms, whether soluble or in suspension, could originate in the aquatic habitat or be contributed from other sources such as soil runoff (Olsen, 1967). Once dissolved in water, soluble forms such as selenate can be subject to subsequent precipitation if the alkalinity of the water is lowered. The lower pH together with poor aeration favors reduction to selenite which will precipitate with ferric hydroxide (Lakin and Davidson,

1966). Such a situation could occur when alkaline spring or surface drainage waters are diluted by less alkaline river or lake water.

Much of the early work concerning the environmental dynamics of Se that influence levels in water was done in the western plains and foothills adjacent to the Rocky Mountains (Williams and Byers, 1935; Byers, 1935; Byers et al., 1938). Selenium first attracted attention in this area because its availability in soil was so great that it interfered with livestock production in some locations (see Appendix 1). The region is largely semiarid, alkaline in character, and supplied with abundant Se-containing cretaceous shales. These shales may outcrop, underly soil and water tables, or have been converted to alluvium. In some places, the shales and alluvium have been carried away entirely by erosion. As a consequence. a mosaic of soil and rock types have been formed which contain variable amounts of leachable Se (Olsen, 1967). Ground water and surface runoff are variously affected, depending on local soil and rock type. This, together with air-borne Se translocation, has resulted in a diverse array of Se-containing springs, streams, lakes, and reservoirs, some of which are subjects of this study.

Information that has been collected on the actual Se levels which occur in water is limited and only a few generalizations are possible. Ocean water exhibits both lower and less variable levels than does terrestrial associated water. The mean value for ocean water is given in most reviews (Lakin, 1973; Bowen, 1966) as 0.09 μg/l with

a range of 0.05 to 0.14 μg/l. In one report, however, values as high as 6.0 μg/l were given (Subcommittee on Selenium, 1976). The expression μg/l (micrograms/liter) is equivalent to the term, ppb (parts per billion). In the case of inland waters, it is not possible to assign a meaningful average level. Values from less than 1.0 to 14 μg/l have been found in United States watersheds and public water supplies. A maximum of 400 μg/l was reported in Colorado river tributaries (Scott and Voegeli, 1961). Ground water can also be quite variable. Levels up to 330 μg/l were found in well water from a seleniferous area in South Dakota. However, it is important to understand that waters from any source with values exceeding 0.5 μg/l are considered unusual and that such levels are associated with ground water and drainage from seleniferous areas (Oldfield, 1974).

Underwood (1971) stated that the levels usually encountered in drinking water are not an important contribution to dietary intake of Se in man or domestic animals. United States government recommendations (Committee on Water Quality Criteria, 1972) for allowable levels in water are set at 10 μg/l for human consumption and 50 μg/l for livestock. These values are considerably greater than those found in most surface waters. Se concentration in water high enough to cause animal poisoning would be very rare (Olsen, 1967).

With regard to effects on aquatic organisms, Se bioassay toxicity studies have been performed. However, they are not directly relevant to this discussion because both the forms and amounts of Se

involved in this work are atypical of the levels found in aquatic habitats. Some representative studies are reviewed in Appendix 3.

The kinds of inquiry which do advance understanding of Se dynamics in aquatic habitats are those which address questions about uptake, turnover, and accumulation of Se in the tissues of aquatic organisms and sediments.

Selenium Levels in Aquatic Organisms and Sediments

Tissue levels of Se have been systematically determined for plant and animal food items of terrestrial origin consumed by man (Appendix 4).

For aquatic species, there have been surveys of naturally occurring Se levels as well as some experimentation designed to determine the nature of Se uptake. Table 1 is a condensation of data arranged by organism type from a variety of reports describing Se levels in marine organisms. The marine plant samples vary between 0.02 and 0.24 μg/g. This fact suggests that marine algae have Se enrichment (bioconcentration) factors ranging up to approximately 9500. These factors are derived by dividing a given algal Se level by the mean sea water level: $[(0.24\ \mu g/g)/0.09\ \mu g/l)]$ x (1000 g/l) = 9450. The expression, 1000 g/l, is necessary because the organism level units expression (μg Se/g) is 1000 times greater than the water level expression (μg Se/l). For whole Mytilus galloprovincialis (clam) as an example, the overall bioconcentration factor (based on a

Table 1. Marine Organism Tissue Selenium Levels

Organism	Tissue	µg Se/g Dry Weight Mean	Range	Location	Reference
Plants	Whole		0.04-0.07	Reine in Lofoten, Norway	Lunde, 1970b
Laminaria caniculata					
Ascophylum nodosium	Whole		0.06-0.09	Reine in Lofoten, Norway	Lunde, 1970b
Marine Algae (various)	Whole		0.12-0.24	Reine in Lofoten, Norway	Lunde, 1970b
Marine Algae (various)	Whole		0.04-0.17	Troud helmsfjord, Norway	Lunde, 1970b
Marine Algae (various)	Whole		0.02-0.12	Baltic Sea	Sandholm et al., 1973
Invertebrates	Whole		1.09-2.43	Baltic Sea	Sandholm et al., 1973
Zooplankton					
Lymata seticulata	Whole	2.69		Port of Monaco	Fowler and Benayoun, 1976
(shrimp)	Viscera	7.06		Port of Monaco	
	Muscle	1.98		Port of Monaco	
	Eye	4.86		Port of Monaco	
	Exoskeleton	1.51		Port of Monaco	
Cranyon Cranyon	Carapace	5.0	1.6-8.3	Belgain Coast	Bertine and Goldberg, 1972
(shrimp)	Tissue	6.1	3.9-15.0	Belgain Coast	
Blue Crab	Meal		0.08-6.70	Not reported	Kiefer et al., 1969
King Crab	Meal		0.34-0.15	Not reported	Kiefer et al., 1969
Mytilus edulis	Shell	0.05	0.02-0.06	Belgain Coast	Bertine and Goldberg, 1972
(mussel)	Soft parts	4.5	3.5-5.6		
Mytilus edulis	Not reported	3.9		Not reported	Lunde, 1970a
Pectin maximus	Not reported	2.6		Not reported	Lunde, 1970a
(clam)					
Mytilus gallo-	Whole	6.10		Port of Monaco	Fowler and Benayoun, 1976
provincialis	Shell	<0.05		Port of Monaco	
(clam)	Mantle	5.26		Port of Monaco	Fowler and Benayoun, 1976
	Gills	7.08		Port of Monaco	
	Viscera	3.22		Port of Monaco	Fowler and Benayoun, 1976
	Muscle	1.91		Port of Monaco	

Table 1. Continued

Organism	Tissue	μg Se/g Dry Weight Mean	Range	Location	Reference
Ommasstrephes sagittatus (squid)	Not reported	3.0		Not reported	Lunde, 1970a
Vertebrates					
East Canadian herring	Meal	1.95	1.30-2.60	Atlantic	Kiefer et al., 1969
Chilean Anchovetta	Meal	1.35	0.84-2.60	Pacific	Kiefer et al., 1969
Tuna	Meal	4.63	3.40-6.20	Not reported	Kiefer et al., 1969
Smelt	Meal	0.95	0.49-1.23	Not reported	Kiefer et al., 1969
Menhaden	Meal	1.93	1.10-3.70	Mississippi	Kiefer et al., 1969
Menhaden	Meal	2.16	0.75-4.2	Atlantic	Kiefer et al., 1969
Gadus morhua (cod)	Various		1.47-2.45	Not reported	Lunde, 1970a
Clupea harengus (herring)	Bone	0.7		Not reported	Lunde, 1970a
	Muscle	1.2			
	Liver	3.7			
	Skin	8.6			
Scomber scomber (mackeral)	Various		1.3-1.4	Not reported	Lunde, 1970a
Makaira indica (Black Marlin)	Muscle	2.2*	0.04-4.3*	Australian Coast	Makay et al., 1975
	Liver	5.4*	1.4-13.5*	Australian Coast	Makay et al., 1975
Fish (various)	Meal	1.94	1.47-2.45	Various	Lindberg, P. in Lakin, 1973
Fish (various)	Muscle		1.09-2.00	Atlantic	Sandholm et al., 1973
Fish (various)	Muscle		0.96-2.45	Baltic	Sandholm et al., 1973
Seal	Meal	0.86		Baltic	Sandholm et al., 1973
Whale	Meal	0.90		Atlantic	Sandholm et al., 1973
Various mammals	Liver		0.60-134*	Atlantic	Koeman et al., 1975
Balaenoptera physolus (whale)	Muscle	0.5		Not reported	Lunde, 1970a
Birds (various)	Liver		2.4-4.6	Atlantic	Koeman et al., 1975
	Brain		0.46-1.1	Atlantic	

*Wet weight values.

water value of 0.09 μg/l) would be approximately 68,000. Note that the mean Se level for ocean water (0.09 μg/l) must be used in these calculations because none of the reports cited in Table 1 included data on the actual levels of Se in the water. Table 1 also includes some information on the internal distribution of Se in marine animals. For instance, there is considerable variation in Clupea harenqus and Mytilus galloprovincialis depending on the tissue involved. The extremely high levels for liver from marine mammals reported by Koeman et al. (1975) were measured in conjunction with a study of Se and mercury co-accumulation. This and other investigations (Ganther et al., 1972) have suggested the possibility that levels of other elements like mercury can influence the extent to which Se is accumulated in marine organisms (See Appendix 1).

With regard to Se levels that have been reported for fresh water species, the reports that have appeared are summarized in Table 2. In a few of these, some attempt was made to relate habitat Se levels with that in organisms.

Lake Michigan zooplankton levels varied from 1.0 to 7.0 μg/g (Copeland, 1970). The pattern of variation was attributed to differential deposition into the lake of wind-borne industrial emissions containing Se. Those areas most remote from the sources of the emissions showed the lowest zooplankton Se levels. Unfortunately, the water concentrations were not reported.

Table 2. Fresh Water Organisms. Tissue and associated water Se levels.

Organism	Tissue/Form	Tissue Se µg/g	Water Se µg/l	Remarks	Reference
Potomogeton sp. (Pondweed)	Whole/NR	15-20	90-160	Sweitzer L. Delta, CO	Barnhard, 1957
Anax sp. Nymphs (Dragonfly)	Whole/NR	3.5-5.0	90-160	Sweitzer L. Delta, CO	Barnhard, 1957
Fish (various	Viscera/NR	0.0-8.0	90-160	Sweitzer L. Delta, CO	Barnhard, 1957
Zooplankton	Whole/NR	1.0-7.0		Lake Michigan	Copeland, 1970
Fish (various)	Muscle/wet	0.1-1.5		New York State	Pakkala et al., 1972
Fish (various)	Muscle/dry	1.86-2.85		Natural waters Finland	Sandholm et al., 1973
Salmo iridiens (trout)	Muscle/dry	0.46-0.90		Culture ponds Finland	Sandholm et al., 1973
Macrophytes (various)	Whole/dry	1.5-2.9		Connecticut	Cowgill, 1974
Fish (various)	Muscle/wet	0.16-0.46		Great Lakes	Jervis et al., 1975
Plants (various)	Whole/dry	0.10-0.75		Tuscany, Italy	Rossi et al., 1976
Zygnema sp. (algae)	Whole/dry	0.6, 0.9	0.15, 0.35	New York State	Gutenmann et al., 1976
Potomogeton pectinatus (pondweed)	Whole/dry	0.3, 3.7[2]	0.15, 0.35	New York State	Gutenmann et al., 1976
Plathemis lydia (Dragonfly nymphs)	Whole/dry	1.5, 4.1[2]	0.15, 0.35	New York State	Gutenmann et al., 1976
Ictaluris nebulosus (Bullhead)	Liver/dry	4.5, 9.0[2]	0.15, 0.35	New York State	Gutenmann et al., 1976
Rana clamitans (tadpole)	Whole/dry	1.5, 4.7[2]	0.15, 0.35	New York State	Gutenmann et al., 1976
Gammarus sp. (amphipod)	Whole/wet	0.61	1.87	Wyoming	Moore, 1976
Catostomus sp. (sucker)	Muscle/wet	0.30-1.62	1.14-13.3	Wyoming	Moore, 1976

Table 2. Continued

Organism	Tissue/Form	Tissue Se µg/g	Water Se µg/l	Remarks	Reference
Catostomus sp. (sucker)	Liver/wet	1.14	1.15	Wyoming	Moore, 1976
Perca sp. (perch)	Muscle/wet	0.59	1.15	Wyoming	Moore, 1976
Perca sp. (perch)	Liver/wet	1.13	1.15	Wyoming	Moore, 1976
Salmo sp. (planted trout)	Muscle/wet	0.09-0.59	0.21-0.76	Wyoming	Moore, 1976
Salmo sp. (planted trout)	Skin/wet	0.04-2.76	0.21-0.76	Wyoming	Moore, 1976
Salmo sp. (planted trout)	Liver/wet	1.71-15.40	0.21-0.76	Wyoming	Moore, 1976
Salmo sp. (planted trout)	Muscle/wet	0.44-3.02	1.15-13.3	Wyoming	Moore, 1976
Salmo sp. (planted trout)	Skin/wet	0.27-4.79	1.15-13.3	Wyoming	Moore, 1976
Salmo sp. (planted trout)	Liver/wet	1.13-71.35	1.15-13.3	Wyoming	Moore, 1976

[1] Wet and dry weight tissue levels are not directly comparable to each other because the degree of hydration for wet samples is variable. NR indicates that the tissue form was not reported.

[2] Se tissue levels separated by comma (,) were associated with the corresponding water Se values also separated by a comma. Thus, for P. pectinatus 0.3 µg/g plant tissue was found in 0.15 µg/l water and 3.7 µg/g tissue was found in 0.35 µg/l water.

Gutenmann et al. (1976) studied ponds contaminated with Se containing fly ash. While only two different water Se levels were involved, there were indications of a correlation between Se amounts in water and organisms.

An investigation of stocked fish mortality in Sweitzer Lake near Delta, Colorado, yielded the tentative conclusion that Se was poisoning fish via the mechanism of food chain bioconcentration (Barnhard, 1957). Water levels varying from 90 to 150 μg/l were reported along with Se levels in organisms ranging between 0.0 to 20 μg/g. State of Colorado authorities have subsequently examined the Sweitzer Lake situation and recommended that fish caught there not be used for food because of the possibility that Se in the fish may pose a health hazard (Associated Press, 1977).

Fish tissue and water Se levels from a region in southeastern Wyoming that contain locally seleniferous areas have been reported in unpublished studies (Moore, 1976). As indicated in Table 2, the Se levels were extremely variable and ranged up to 71.35 μg/g wet weight for trout liver. Obviously, very unusual tissue levels were found and the data suggest the possibility of a correlation between water and tissue Se levels.

As is the case for the marine habitat, freshwater inhabitants exhibit several thousandfold bioconcentration factors. Using the Gutenmann et al. (1976) data associated with 0.35 g Se/l water as an example, factors ranging from 2571 for Zygnema sp. to 13,429 for

Rana clamitans were observed. Selected fish tissues such as liver exhibited Se levels which would result in even higher calculated bioconcentration factors.

Experimental approaches to the question of Se uptake and bioconcentration have also been undertaken. Radioactive ^{75}Se as selenite was used in a 52-day sea water aquaria experiment (Fowler and Benayoun, 1976). Shrimp (Lymata seticulata) and mussels (Mytilus galloprovincialis) were used to evaluate the relative Se contribution to tissue from food versus that from water. The authors concluded that while Se uptake from water occurred, its contribution was minor compared to that accumulated from food. The food in this case was mussels that had taken up ^{75}Se-selenite which were in turn fed to shrimp in chopped form.

Sandholm et al. (1973) performed very short-term (three-day) fresh water aquaria experiments to assess Se exchange between water, algae, zooplankton and fish. Using radioactive tracer techniques, they concluded that ^{75}Se-selenomethionine in water was rapidly absorbed by algae while ^{75}Se-selenite was not. Uptake for fish seemed to occur via the food chain and not directly from the water.

Gissel-Nielsen and Gissel-Nielson (1973) did longer term (eight weeks) work on ^{75}Se-selenite uptake in leeches (Glossiphonia complanata), caddis fly larvae (Limnophilus flavicornis) and water thyme (Helodea canadensis). Although they did not directly measure Se levels in tissues, they did attempt to assess enrichment factors in

aquaria systems at two augmentation levels of ^{75}Se-selenite in water (1.1 and 14.3 μg/l were added). They concluded on the basis of radio-active counts that Se enrichment factors at these concentrations for thyme and caddis fly larvae were similar to the 1000-fold enrichment of unaugmented aquaria system which contained 0.2 μg/l of native Se. The Se enrichment factors for leeches in the augmented aquaria reached about 4000 by the end of the experiment versus 10,000 for the unaugmented system. For a given species, similar bioconcentration factors over a range of Se water levels is an indication that tissue levels may bear a direct relation to the Se levels of the water.

Selenite accumulation in algae was included in an extensive study of trace element uptake in mixed algal growths cultured on glass slides (Patrick et al., 1975). At flowing water levels of 5000 μg/l selenite, the algae accumulated 4000 μg/g (800-fold bioconcentration). At 10,000 μg/l, the algal level was 5000 μg/g (500-fold). The factor fell off sharply at higher water levels of selenite. These results are interesting because they suggest the possibility that algae exhibit at least several hundred-fold bioconcentration factors at dissolved Se water levels up to and considerably beyond those found in natural habitats.

Understanding of Se dynamics in aquatic systems also requires that sediment levels be examined. Sediments are a complex mixture of substances from both biotic and abiotic sources. Similarly, chemical transformations which occur in sediments may or may not

be mediated by biological processes (Wetzel, 1975). Depending on the character of the water body and the elements in question, sediments can be either a repository of available forms or a sink for immobilized forms of elements being cycled through aquatic systems. Sulfur, nitrogen and phosphorus (to name a few) have been extensively studied in this regard. Selenium has not. Appendix 2 outlines what has been reported regarding microbial transformations of Se and naturally occurring sediment levels have been measured in connection with one of these studies. Investigations of 12 Ontario Lake sediments (Chau et al., 1976) revealed a wide range of values (0.48 to 20.48 μg/g) but nine were less than 1 μg/g dry weight. In another report not dealing with microbial transformation, Wisconsin workers (Wiersma and Lee, 1971) examined 12 lake sediments and found Se levels ranging from 1 to 3 μg/g dry weight. A recent study involving the levels of many elements in sediments from the Great Lakes has also been performed (Jervis et al., 1975). In the uppermost layers, Se levels ranged from 0.16 μg/g in Lake Huron to 1.8 μg/g in Lake Ontario. There was also evidence of nearly ten-fold greater Se levels in more recent sediments as compared to those deposited several decades ago. Unfortunately, none of these investigations correlated sediment level Se with any other aspect of the aquatic system such as water, plankton, or macrophyte Se levels.

Before concluding the discussion on Se accumulation by aquatic organisms, it should be noted that none of the reports reviewed thus

far have discussed the mechanisms by which Se compounds enter into and are assimilated by aquatic organisms. In the case of algae, this poorly understood process is very important because algae may be principally responsible for incorporating Se into the aquatic food chain. As indicated in Appendix 1, this process is not too well understood in terrestrial plants either. As with plants on land, it may be that Se uptake is an unavoidable consequence of the requirement for analogous sulfur compounds. Active transport uptake antagonism between Se and sulfur compounds has been detected in Chlorella vulgaris as well as in Scenedesmus sp. (Shrift, 1973). Aquatic plants concentrate sulfur as much as 1000-fold over the levels in the surrounding water where it exists primarily as sulfate (SO_4^{-2}) (Wetzel, 1975). This is comparable to the reported Se bioconcentration levels discussed in the previous paragraphs.

The question as to what forms of Se are most readily metabolized by aquatic plants has also received some attention (Butler and Peterson, 1967) in a study using the monocot duckweed, Spirodela oligorrhiza. Radioactive ^{75}Se labeled selenite, selenate, and colloidial Se were all actively taken up from culture solution by the plants. Selenite was taken up most readily and much of it was metabolized into organic forms such as protein bound selenomethionine. Colloidial Se was also converted into many other forms after absorption. Selenate was comparatively more resistant to metabolic alteration after being

absorbed. It was suggested that before conversion to organic form, selenate must first be reduced to selenite and that this process was rate limited. These investigators also concluded that the pH (5 versus 7.2) of the culture media had no significant effect on Se uptake and assimilation.

There seems to be no reported information on the mechanisms involved in Se uptake from water or food. The reports discussed above and those cited elsewhere which deal with coaccumulation of mercury and Se from ocean water do not deal with the question of how Se is extracted from water or food by aquatic organisms.

It is clear from the work reported to date that extensive bioconcentration of Se in the tissues of aquatic organisms does occur. The resulting levels vary from species to species and there is interest in the significance of the accumulation process. There is also good reason to suspect that Se levels in aquatic organisms are influenced to some extent by the availability of Se in their habitat. Moreover, the levels of other aquatic constituents such as sulfate may modify the extent to which selenium is bioconcentrated.

CHAPTER II

FIELD STUDY MATERIALS AND METHODS

Selection of Study Sites and Organisms

A wide range of native Se water levels was sought during this study. In order to locate potential sampling locations, reported data on Colorado and Wyoming soil levels were evaluated (Byers, 1935; Byers, 1936; Byers et al., 1938) as well as information from studies of contaminants in Colorado streams (Williams and Byers, 1935; Scott and Voegeli, 1961; Wentz, 1974). Geologic and topographic maps of Colorado (USGS, 1975; USGS, 1973) and Wyoming (USGS, 1955; USGS, 1967) were used to find probable locations of weathering Se-rich cretaceous shales that might influence water levels of the element. In addition, William G. Hepworth of the Wyoming State Game and Fish Laboratory in Laramie, Wyoming and Lloyd K. Hazzard of the Colorado State Fish and Game Department Regional Headquarters in Montrose, Colorado, were interviewed concerning their knowledge of water Se levels. As a consequence of these inquiries, four areas were chosen for a survey of selenium levels. Three of these were in Colorado in the vicinity of Fort Collins, Grand Junction and LaJunta. The fourth was in the vicinity of Laramie, Wyoming.

Using the procedures described in the following Section, survey samples of water, sediment, and biota were taken from various sites (lakes, ponds, and reservoirs) and analyzed for Se. Electrical conductivity was also measured at each site. Sites for which Se levels were determined are given in Table 3.

The following criteria were used to select organisms that would receive major study emphasis. The species had to have a high probability of being indigenous to the specific site where collected, be found in at least three sites differing significantly in water Se level, be collectible in quantity sufficient for analysis and be readily separable from other types of organisms and contaminating material such as sediment. Fish stocked from hatcheries do not meet the criteria because they are not indigenous to the site where caught. Their Se levels will have been partly influenced by thehabitat where they were hatched and raised. Zooplankton also does not fit the criteria because it represents a heterogeneous mixture of species which can be expected to vary considerably with both time and location. However, some zooplankton was analyzed in the course of this research because it was used in the experimental studies of Se uptake by fish. Microscopic phytoplankton was also not included because it too is a mixture of organisms. Moreover, it is very difficult to reliably separate sufficient quantities of such organisms from suspended or bottom sediment particles having sizes and densities which are similar to

Table 3. Study Site Names, Selenium Levels, and Locations.

Site No.	Site Name	Location	Mean Selenium µg/liter	Grid Reference			County Hiwy Map Source		
				Range	Township	Sec.	County	Sheet #	Date
1.	Alsop Lake	Laramie, WY	0.3	R75W	T16N	S3NW	Albany	1	1967
2.	Park Creek Reservoir*	Wellington, CO	0.3	R69W	T10N	S7S	Larimer	2	1958
3.	Meeboer Lake*	Laramie, WY	0.3	R75N	T14N	S4N	Albany	1	1969
4.	Diamond Lake*	Bosler, WY	0.3	R74W	T19N	S23N	Albany	3	1969
5.	Big Thompson Pond	Loveland, CO	0.6	R68W	T5N	S15W	Larimer	3	1958
6.	Lake Hasty*	Hasty, CO	0.7	R49W	T23S	S5S	Bent	1	1966
7.	Galett Lake*	Laramie, WY	0.8	R75W	T15N	S24S	Albany	1	1969
8.	Eight Mile Lake	Laramie, WY	0.9	R74W	T15N	S21W	Albany	2	1969
9.	Seven Mile Lake	Laramie, WY	1.0	R74W	T15W	S2N	Albany	2	1969
10.	Smith Lake	Wellington, CO	1.5	R68W	T9N	S20SW	Larimer	2	1958
11.	Wellington State Pond*	Wellington, CO	1.7	R68W	T9N	S26S	Larimer	2	1958
12.	Timber Lake*	Cheraw, CO	2.1	R54W	T21S	S25SE	Otero	1	1963
13.	Mac Mesa Reservoir*	Mack, CO	2.2	R3W	T2N	S5N	Mesa	1	1962
14.	Nine Mile Lake	Laramie, WY	2.4	R74W	T15W	S29N	Albany	2	1969
15.	Ordway Reservoir	Ordway, CO	2.4	R57W	T21S	S2E	Crowley	1	1964
16.	Blue Lake	Las Animas, CO	2.5	R52W	T21S	S5N	Bent	1	1953
17.	Wellington Reservoir #4	Wellington, CO	3.6	R68W	T9N	S29SW	Larimer	2	1958
18.	Cobb Lake*	Wellington, CO	3.8	R68W	T8N	S20N	Larimer	2	1958
19.	Highline Reservoir*	Mack, CO	4.2	R3W	T2N	S5NW	Mesa	1	1962
20.	East Allen Reservoir*	Medicine Bow, WY	4.8	R78W	T22N	S18SW	Carbon	5	1962
21.	Lake Henry*	Ordway, CO	5.9	R56W	T21S	S6N	Crowley	1	1964
22.	Miller's Lake*	Wellington, CO	6.0	R69W	T10N	S20N	Larimer	2	1958
23.	Twin Buttes Reservoir*	Laramie, WY	7.6	R75W	T15N	S24S	Albany	1	1969
24.	Colorado Route 71 Pond	Ordway, CO	7.7	R56W	T18S	S20NW	Crowley	1	1964
25.	Duck Lake*	Fort Collins, CO	9.1	R68W	T6N	S17S	Larimer	3	1958
26.	Lake Meridith	Ordway, CO	9.1	R56W	T21S	S20E	Crowley	1	1964
27.	Sweitzer Lake*	Delta, CO	9.4	R95W	T15S	S28SW	Delta	1	1963
28.	Cheraw Lake	Cheraw, CO	12.0	R54W	T22S	S36W	Otero	1	1963
29.	Desert Reservoir*	Grand Junction, CO	12.5	R2E	T2S	S30W	Mesa	2	1962
30.	Larimer Highway 9 Pond*	Fort Collins, CO	15.8	R68W	T7N	S17SE	Larimer	2	1958

*Sites which were employed in the detailed study of selenium levels.

those of the organisms. The following sources were used as references for the identifications of organisms: Pennak (1953), Usinger (1956), Baxter and Simon (1970).

Sample Collection and Preparation

Samples of water, sediment and biota were obtained at various times during March 1976 through April 1977 for survey purposes. Twice-monthly serial samples of the plains killifish, Fundulus kansae, were taken at Twin Buttes Reservoir (see Table 3) during August through October 1976 for the purposes of determining if tissue Se levels per unit weight increased during rapid growth.

Samples of water, sediments and biota were again taken at monthly intervals during the period April through June 1977. Analytical results from these samples were the principal source for the Se level correlations reported in the results and discussion section.

Invertebrates (except zooplankton) were netted or picked from plants or bottom sediments. Fish were netted with dipnets, seines, or gillnets.

Plants were taken by hand or by means of a hook dragged along the bottom. Sediments and associated organisms such as Chironomus larvae were obtained with an Eckman dredge. Water was obtained by uncapping a plastic bottle below the surface in site areas judged typical of the biota collection location.

All collected materials were placed in capped plastic containers and transported in ice-containing chests to the laboratory. At the laboratory, invertebrates, small fish, and tissue dissected from large fish were immediately cleared of debris by a tapwater rinse and preserved by freezing at -15°C. Plants and sediments were immediately preserved by oven drying in shallow glass containers at 40°C for 48 hours. Water samples were preserved by refrigeration at 4°C.

Prior to weighing, sediments were ground in a porcelain mortar and sieved through a 0.5 mm mesh plastic screen. Plants were ground in a stainless steel Wiley mill (Arthur H. Thomas Company), fitted with a #40 mesh screen. The frozen fish and invertebrates were dehydrated and prepared for weighing by lyophilizing (freeze drying) using a Virtis brand dry-ice-acetone chilling bath and vacuum manifold. Vacuum was provided by a Welch Duo Seal Model 1400 pump and maintained for eight hours. Lyophilizing vessels were 25-ml Erlenmeyer flasks (Corning #26500). The freeze-dried materials were then crumbled with a spatula blade and placed in tared 16 x 150 mm plastic digestion tubes (Falcon #2045). Sample weights ranged between 0.01 and 0.3 g depending on the size and availability of sample material. In most cases, several individual organisms had to be combined to provide a large enough sample weight. Duplicates were always prepared.

Digestion procedures adapted from Davies and Adrian (1975) were employed. For the freeze-dry samples, one ml of concentrated

nitric acid (HNO_3) was added to each tube and followed 24 hours later by 1 ml of 30 percent hydrogen peroxide (H_2O_2). After six hours, the tubes were placed in a 40°C water bath for 24 hours. The tubes were kept lightly capped throughout the digestion to avoid excessive pressure buildup. The digested mixture was then filtered through 5.5 cm Whatman #541 paper into another 16 x 150 mm tube. Volume was brought to 16 ml using glass distilled/deionized water. Duplicate blanks subject to all the steps above except sample introduction were also prepared. For the oven-dried plant and bottom sediments, a modification of the above procedure was employed. Samples were placed into tared 25-ml Erlenmeyer flasks. Sample weights were approximately 0.50 g. Five milliliters of concentrated HNO_3 was added and the flasks were heated 24 hours on a 60°C hot plate. One additional milliliter of HNO_3 and 2 ml 30 percent H_2O_2 were then added and the mixture was heated for an additional 24 hours. The flasks were kept lightly stoppered throughout to permit refluxing. The digested mixture was filtered and diluted as described above. Duplicate blanks were also prepared.

Water samples were pretreated for analysis using the acid-persulfate method (Goulden and Brooksbank, 1974). Within three days after collection, 50-ml portions of sample were vacuum filtered free of suspended matter (Sartorius 0.45 μm membrane filter and Millipore filter holder) and placed into a 125-ml Erlenmeyer flask. To this was added 1 ml of concentrated hydrochloric acid (HCl) and

5 ml of 2 percent potassium persulfate ($K_2S_2O_8$). The mixture was boiled for 20 minutes and then restored to 50 ml with glass distilled/deionized water. In samples known to contain very low levels of Se (<0.5 ug/l), the sample was concentrated three-fold by starting with a 150-ml water sample with subsequent restoration to 50 ml. Samples containing very high levels (>10 μg/g) were diluted with distilled/deionized water.

Analytical Procedures

Biota and sediment samples prepared as described above were analyzed for Se with an atomic absorption spectrophotometer (AAS) equipped with a heated graphite atomizer (HGA) also known as the carbon rod furnace. Trial determinations were made with a Perkin-Elmer 503/AAS and HGA/2000. Subsequent analysis was done with a Perkin-Elmer 306/AAS and HGA 2100. Both instruments were equipped with deuterium arc background correction and absorbance peak readers.

Nickel was added to the samples prior to analysis to improve accuracy (Ihnat, 1975; Brodie, 1977). The addition of nickel converts the selenate in the digested sample to a nickel-selenium complex (thought to be nickel selenide). In this form the sample can be charred in the HGA at a higher temperature (1300°C versus 400°C) which more effectively removes interfering substances. One milliliter of 5 percent nickel as nickel nitrate $[Ni(NO_3)_2 \cdot 6H_2O]$ was added to 4 ml of

digested/diluted sample in a 13 x 100 plastic tube (Falcon #2027) using glass volumetric pipets. An Eppindorf pipet and plastic tips were used to inject 50 µl of nickel-complexed samples into the HGA. Two replicate injections were made for each sample. Table 4 describes the instrument parameters used for analysis. Peak absorbance readings were converted to µg Se/g sample by the following formula:

$$\mu g\ Se/g = \frac{(\text{sample absorbance})\ (\text{dilution factor})\ (\text{mg Se/l standard})}{(\text{standard absorbance})\ (\text{sample weight in g})}$$

Standards were prepared from Se shot (Ventron #00290) dissolved in concentrated HNO_3 according to the method described in Perkin-Elmer (1976). Standards of .02, .04 and .06 mg/l were employed.

This analytical procedure and the digestion technique described earlier were checked with National Bureau of Standards Reference Material. Internal consistency of the method over a wide sample weight range was also checked using homogeneous samples of plankton and fish tissue collected from the study sites. The results of these validation procedures are given in Chapter IV.

Se levels in water were determined by the hydride generation AAS technique described by Goulden and Brooksbank (1974) and Pierce et al. (1976). The analyses were performed by the Colorado State University Analytical Chemistry Facility. In the procedure, which employed a Varian 1200 AAS and automated attachments for

Table 4. Atomic Absorption Spectroscopy (AAS) Parameters for Selenium Analysis

Perkin Elmer (P.E.) 503 and 306 Atomic Absorption Spectrophotometers	
Lamp Type	P. E. Electrodless Discharge Lamp #303-6263
Lamp Power	6.0 watts
Monochronometer Wave Length	194.5 nm (ultraviolet range)
Monochronometer Slit Width	0.7 nm (setting #4)
Atomization Method	Heated Graphite Atomizer
Perkin Elmer 2000 Heated Graphite Atomizer[1]	
Drying Stage	100°C for 50 seconds
Charring Stage	1400°C for 40 seconds
Atomization Stage	2700°C for 15 seconds
Purge	Argon (3 flow units) Auto-interrupt
Typical Absorbance (minus blank) for 50 µl of .010 mg Se/l Standard is approximately .070 absorbance units[2]	
Perkin Elmer 2100 Heated Graphite Atomizer[1]	
Drying Stage	100°C for 40 seconds
Charring Stage	700°C for 30 seconds
Atomization Stage	2500°C for 12 seconds
Purge	Argon (15 flow units) continuous
Typical Absorbance for 50 µl of .020 mg Se/l standard is approximately .040 absorbance units.	

[1] Samples must contain added nickel (1% w/v nickel nitrate) to prevent Se volitization during the charring stage.

[2] The P. E. 2000 Heated Graphite Atomizer produces a peak absorbance of .010-.015 absorbance units when a Se-free blank is injected. Note that the expression, .010 mg Se/l is equivalent to 10 µg Se/l or 10 parts per billion.

aspirating and processing the sample, the selenate (SeO_4^{-2}) in 10 ml of pretreated water is first reduced to the selenite (SeO_3^{-2}) form by a potassium iodide-stannous chloride mixture. It is then converted to a volatile hydride form (H_2Se) with atomic hydrogen generated by an aluminum powder-HCl reaction. The hydride is then swept into the AAS light path by an air-argon stream. While in the path, the stream is confined by an electrically heated silica tube. Accuracy of the determinations was checked by using the Se standards that were employed in conjunction with the biota and sediment analyses.

The hydride method was chosen for water samples because it is comparatively free of interferences by other ions in the samples such as magnesium (Mg^{+2}) and sulfate (SO_4^{-2}). These ions were present at high concentrations in many of the waters analyzed. Extensive trials were performed with AAS-HGA water analysis techniques employing methods designed to avoid these interferences (Henn, 1975). However, this method was not found to be as satisfactory as the AAS-hydride technique. See Appendix 5 for a discussion of water analysis by the AAS-HGA method.

In addition to being subjected to Se analysis, sediments and water were also analyzed for other components. In the case of sediments, organic carbon levels were measured. For water, the additional determinations consisted of sulfate, hardness, and conductivity measurements.

Sediment organic carbon levels were estimated according to a procedure modified from the Walkley-Black method given by Allison (1965). In this determination, up to 2 g of ground and sieved sediment were treated with 10 ml 1 Normal potassium dichromate ($K_2Cr_2O_7$) and 20 ml concentrated sulfuric acid (H_2SO_4) in a 250-ml Erlenmeyer flask. After the mixture was allowed to cool for 30 minutes, 200 ml of distilled/deionized water was added and the mixture was titrated with 0.5 Normal ferrous sulfate ($FeSO_4 \cdot 7H_2O$). The endpoint was a change in color from blue-green to maroon. Blanks prepared as described above but without sediment were also titrated. Organic carbon in the sediment is calculated from the volume required to titrate the samples (sample ml) and blanks (blank ml) using the following formula:

$$\text{g organic carbon/g sediment} = \frac{(\text{blank ml} - \text{sample ml})\ (.002\ \text{g/ml})}{\text{sediment sample weight in grams}}$$

It should be noted that this is a formula which has been derived empirically for soils and thus cannot be expected to give an absolute value for sediment. However, relative variations in carbon levels from sample to sample are detectable by this method and thus is sufficient for correlating sediment Se and organic carbon levels.

Sulfate levels in water were measured by the turbidimetric method (APHA, 1976) using a spectrophotometer, reagents, and instructions supplied by Delta Scientific (Delta, 1972). In this

procedure, barium chloride (Delta #R-116) and a conditioning reagent (Delta #R-115) were used to form a finely divided, suspended barium sulfate precipitate. The amount of precipitate was measured in an absorption cell at 420 nm with a Delta #260 photometric analyser. A calibration curve supplied with the instrument was used to convert absorption percentage to mg sulfate/l water. The accuracy of the calibration curve was checked with standards made from anhydrous sodium sulfate (Na_2SO_4) ranging in sulfate concentration from 10 to 50 mg/l. Samples were diluted with distilled/deionized water so that they also fell into the 10 to 50 mg/l range.

Hardness levels (principally the cations, magnesium and calcium) in water were measured by the EDTA (ethylene diamine tetraacedic acid) titrametric method (APHA, 1976) using reagents and instructions supplied by the Hach Chemical Company (Hach, 1975). In this procedure, buffer solution (Hach #424 - Hardness I) and a powdered reagent mixture containing indicator (Hach #851 - Manver II) were added to the sample which was then titrated to endpoint with EDTA (Hach #205 - 0.02 N Titraver). The procedure was checked against standards made with calcium carbonate ($CaCO_3$) having hardness levels in the 50 to 150 mg/l range. Samples were diluted with distilled/deionized water so that they also fell into this range.

Electrical conductivity of water at the study sites was measured with a Yellow Springs Instrument Company Model 37 Salinity-Conductivity-temperature meter according to the instructions

provided by the manufacturer. The instrument calibration was checked with standards made from potassium chloride (KCl) prepared according to the method given in APHA (1976). Conductivity, measured in μ siemans/cm^2 (also known as μmhos/cm^2), was used as an indication of the magnitude of total dissolved solids (TDS) measured in mg/l. Tables are available (APHA, 1976) which relate the two values.

CHAPTER III

EXPERIMENTAL METHODS AND MATERIALS

Experimental Design

The experimental portion of this research was designed to discriminate between the effects of two variables which could influence fish tissue Se levels. One possible source of Se is uptake from food by the gut. The other is uptake directly from water by exposed surfaces such as skin and gills. To test the effects of the food and water variables, fish were raised from fry in aquaria filled and periodically renewed with water from sites which differened in Se concentration. The fish were supplied food rations with different levels of Se content. An experimental array was thus formed wherein fish in any one of the aquaria would be influenced by one of several food Se levels and one of several water Se levels. In this way, each fish served as an experimental unit subject to a specific treatment type (see Figure 2). After fish growth occurred, data on tissue Se levels were used to assess the relative affects of food and water using analysis of variance techniques.

General Experimental Procedures

Tanks used to contain the fish were kept covered by thin plastic film and subjected to identical seven-day cycles of cleaning and water

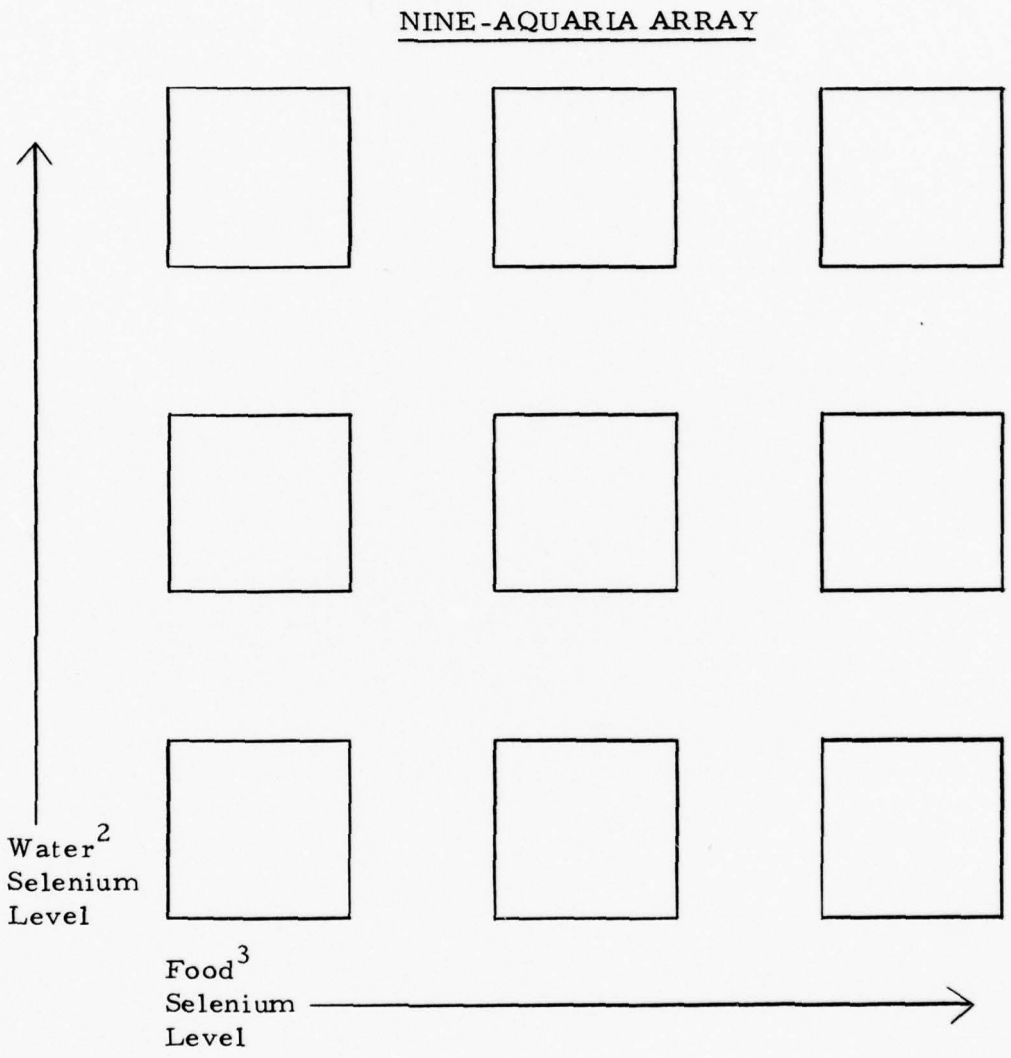

Figure 2. Tissue selenium experiment.[1]

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[1] Fish fry netted from one of the study sites.

[2] Obtained from sites of differing selenium levels.

[3] Mixtures of commercial fish food and indigenous plankton.

renewal. Aeration was supplied by a Silent Giant diaphragm pump via plastic tubing and sintered glass bead aerators. The aerators were cleaned weekly with 10 percent HCl and tapwater rinse. At the time of water renewal, the tanks were scrubbed to remove algal and salt crusts. Room illumination was continuous and supplied by conventional fluorescent bulbs. Room temperature was 20°C.

Water used to support the fish was transported from three sites (Wellington, R. #4, Miller's R., and Twin Buttes R.; see Table 3) in 20-liter plastic jugs. At the time of water collection, care was taken to prevent contamination with bottom sediments. Prior to use in the tanks, the water was allowed to reach 20°C and then was strained through a 64 μm mesh nylon net to remove zooplankton and debris. Both the water being used to renew the aquaria and the water that had been in use seven days were sampled each time the water was renewed. Twenty-five milliliter aliquots from each of these samples were then combined to form composites which were refrigerated at 4°C for later analysis by the methods described in Chapter II.

Fish for each experiment were netted as fry with dipnets from shallow schooling areas in one of the lakes mentioned in the above paragraph and transported to holding aquaria in the laboratory. The fish were then randomly assigned to the experimental tanks. Fish dying during the first week were replaced from the holding aquaria. Several fish from the holding tank were also frozen for later analysis. At the time of tank cleaning and water renewal, fish were captured with

a nylon net and transferred to a renewed tank. At the conclusion of the experiments, the fish were frozen for analysis.

Fish rations were prepared by mixing equal amounts of weighed commercial tropical fish food (Tetra Growth Food produced by Tetra Werka, W. Germany) and freeze-dried plankton. Food was prepared approximately every ten days and each tank was allocated the same weight of food. An amount of food equal to one-half of the tank ration was allocated to a container reserved for serial composite food samples. The composites were subsequently analyzed in duplicate for Se content using procedures described in Chapter II. Fish were fed a portion of their allocated ration twice daily. Aeration was stopped during feeding. Each tank received the same total ration over the duration of the experiments.

The plankton used for preparing the food was obtained by trolling at low speed with a 64 μm mesh nylon net held just below the surface (Wildco student plankton net). The plankton was placed in capped plastic containers and transported in ice-containing chests to the laboratory where it was immediately partially dried by vacuum filtration. A Gast Model 0211-V45N pump was used in conjunction with an 11-cm Buchner funnel and Whatman #541 paper. Most water and the small amount of plankton it contained were decanted away prior to filtration. This procedure yielded 0.5 by 11.0 cm cakes of semi-dry plankton (principally cladocerans and copopods) which were immediately frozen at -15°C. Dehydration of the plankton was

accomplished by cutting the frozen cakes into 0.5 x 0.5 cm cubes which were then processed by the freeze-dry procedure described in Chapter II. The freeze-dried cubes were then crumbled prior to weighing and mixing with the commercial fish food.

Plains Killifish Experiment

Plains killifish (Fundulus kansae) fry (measuring 1.5 - 2.0 cm) from Twin Buttes Reservoir were kept for 90 days on two ration types in the three water types. This resulted in a three by two, six-tank array. One ration was a commercial food-Twin Buttes plankton mixture. The other was a commercial food-Wellington Reservoir #4 mixture. Each of the six four-liter glass jars was initially stocked with ten fish. A total of 3.6 g of ration was supplied to each tank.

Fathead Minnow Experiment

Fathead minnows (Pimephales promelas) fry (measuring 1.5 - 2.0 cm) from Miller's Reservoir were kept for 90 days on three ration types in the three water types. This constituted a three by three, nine-tank array. The rations were as follows: pure commercial food, a commercial food-Twin Buttes Reservoir plankton mixture, and a commercial food-Wellington Reservoir #4 mixture. Each of the nine tanks was initially stocked with 12 fish. A total of 3.6 g of ration was supplied to each tank. This experiment was conducted with four-liter glass jars for the first 30 days. The fish were

then transferred to 10 liters of water in 40-liter rectangular all-glass aquaria.

CHAPTER IV

RESULTS AND DISCUSSION

Verification of Analytical Procedures

The accuracy of the analytical method for Se was verified satisfactorily. National Bureau of Standards Reference Material #1577 (Bovine Liver) containing 1.1 ± 0.2 μg Se/g (NBS, 1972) was analyzed four times during the course of the Se determinations. The values obtained were 1.1, 1.0, 1.0, and 1.0 μg Se/g. The degree of internal consistency over a wide weight range for two different field sample materials is indicated in Table 5. Note that the precision of these results is expressed in tenths of a microgram and that there is some variation in the accuracy. Part of the variation could be due to non-homogeneity in the ground and mixed materials from which the samples were obtained. Moreover, these features tend to be inherent in analytical work of this kind as indicated by the published National Bureau of Standards values for reference materials which also exhibit low precision and some expected variation in accuracy. It should be noted that National Bureau of Standards Reference Material #1571 (orchard leaves) containing 0.08 ± 0.01 μg Se/g (NBS, 1971) fell below the measurable range for the procedures used in this study.

Table 5. Comparison of Selenium Analytical Results for Samples of Different Weight

	Plankton[2]							
Sample weight g	0.0127		0.0104		0.0803	0.0826	0.5053	0.5085
µg Se/g sample	—[1]		—[1]		8.4	8.7	9.0	9.1
	Pimephales promelas meal[3]							
Sample weight g	0.0063	0.0069	0.0107	0.0097	0.0487	0.0501	0.0886	0.0853
µgSe/g sample	8.5	7.2	10.0	9.0	8.1	9.6	8.8	9.4

[1] Below detection level due to low weight of sample.

[2] Obtained from Wellington Reservoir #4 in August, 1976.

[3] Obtained from Miller's Lake in July, 1976. Ten fish, 2-4 cm in length, were ground into a meal.

Field Study Results

The sites employed in the field sampling portion of this study are identified by an asterisk (*) in Table 3. The analytical results for the samples from these sites exhibit definite trends of correlation between organism Se levels and Se levels in water and sediment. That portion of the data suitable for statistical correlation-regression analysis is summarized in Table 6. The ranges of Se levels in the various materials analyzed are evident in this table as well as an apparent lack of relationship between Se levels in water, sediment, and organisms when compared to water levels of sulfate, hardness, and conductivity. A more detailed tabulation of these data is given in Appendix 6.

Least squares linear regression coefficients of determination (R^2) associated with the variables mentioned above were derived by machine computation (Nie et al., 1972). Those variables which exhibited significant coefficients at the $\alpha < 0.01$ level with any of the organisms sampled during the study were water Se level, organic sediment Se (μg Se/g sediment carbon), and sediment Se level. The coefficients are given in Table 7. A statistically significant R^2 value can be thought of as being equivalent to the proportion of variability in the dependent variable (such as organism Se level) that is explained by the variability of the independent variable (such as water Se level). The variables that did not correlate significantly with organism Se level were the water parameters, sulfate, hardness, and conductivity.

Table 6. Selenium Levels Subjected to Correlation-Regression Analysis[1]

		1*	3	4	6	7	11	12	13	18	19	20	22	23	25	27	29	30
Water parameters	Hardness mg/l	80	3800	298	1987	953	2567	615	263	2259	290	953	510	5725	1873	1325	715	958
	Conductivity μSiemans/cm	143	11835	767	4867	1683	3983	1450	1033	3433	1133	4483	900	12000	6667	3383	1625	1683
	Sulfate mg/l	5	8222	333	3650	1117	3533	713	170	2783	212	1950	483	9611	5033	2333	950	967
	Selenium μg/l	0.3	0.3	0.3	0.7	0.8	1.7	2.1	2.2	3.8	4.2	4.8	6.0	7.6	9.1	9.4	12.5	15.9
Invertebrate Se	Hyalella azteca	--	2.3	1.6	--	2.9	4.0	--	--	--	4.3	11.4	7.6	11.3	--	--	18.4	22.5
	Astacidae[2] μg/g	1.2	--	--	--	--	5.4	--	4.7	--	10.1	--	11.3	--	--	20.0	23.3	36.8
	Cenocorixa sp. μg/g	2.3	4.2	2.3	4.2	4.2	5.9	7.3	--	8.4	--	11.0	9.9	15.5	14.4	--	29.4	--
	Coenagrionidae[3] μg/g	--	3.1	1.8	11.1	4.4	7.7	--	11.2	--	28.4	18.7	15.8	18.4	21.9	45.1	55.0	53.3
	Chironomus sp. μg/g	--	2.5	--	--	--	9.0	7.7	--	--	15.3	--	18.8	34.2	23.0	26.7	58.2	59.3
Fish Se	Fry (mixed) μg/g	0.4	--	1.6	4.6	2.1	7.7	--	--	--	--	--	14.1	21.9	--	25.7	60.6	67.2
	Fundulus kansae μg/g	--	7.7	--	4.6	--	5.0	--	--	--	--	--	--	23.1	--	31.9	--	57.4
	Pimephales promelas μg/g	2.1	--	2.1	--	--	--	--	--	--	--	--	11.0	34.5	--	79.0	--	--
	Etheostoma exile	--	--	--	--	2.1	--	--	--	--	--	36.3	23.0	41.9	--	--	--	--
Plankton Se	Mixed species μg/g	--	3.4	1.8	7.9	3.3	4.4	5.8	7.7	--	7.7	11.0	--	15.4	20.8	42.5	31.3	--
Algae Se	Mixed filamentous species μg/g	--	--	0.35	--	0.05	--	2.3	--	7.6	--	3.0	4.6	7.8	--	6.5	--	20.9
	Chara sp. μg/g	--	0.1	0.1	--	0.3	--	--	--	--	16.8	--	--	--	--	14.2	8.8	13.7
Sediment Se	μg/g	--	0.3	--	--	2.8	3.3	0.3	1.8	4.3	1.2	41.0	44.0	10.8	2.1	6.5	15.4	47.3
	μg/g Organic carbon[4]	--	9.4	--	--	48.7	9.8	46.2	87.5	175.5	77.4	114.1	266.7	346.8	155.6	586.3	902.9	1211.5

[1] Mean levels given here are extracted from the more detailed tabulation of analytical results given in Appendix 6.

[2] Composite samples analyzed possibly contained one or more of the genera, Astacus and Orconectes.

[3] Composite samples analyzed possibly contained one or more of the following genera: Enallagma, Ishnura, Lenocorrhina.

[4] The expression μg/g sediment carbon is referred to as "organic sediment Se" in subsequent tables and discussion.

* Site numbers (locations given in Table 3).

Table 7. Organism Selenium Level Coefficients of Determination (R^2)[1]

Organism	Water Se	Organic Sediment Se	Sediment Se
Hyalella Azteca	0.94	0.90	0.75
Astacidae	0.97	0.96	0.83
Cenocorixa Sp.	0.92	0.90	0.77
Coenagrionidae	0.84	0.81	0.47
Chironomus Sp.	0.91	0.89	0.67
Fish Fry	0.92	0.95	0.70
Fundulus kansae	0.98	0.99	N. S.
Pimephales promelas	N. S.	N. S.	N. S.
Etheostoma exile	N. S.	N. S.	N. S.
Plankton	0.78	0.73	N. S.
Filiamentous Algae	0.85	0.88	0.90
Chara Sp.	N. S.	N. S.	N. S.

[1] Each R^2 value given is the one calculated for the Se levels in the corresponding row and column variables. Thus the coefficient of determination for Fish Fry Se and Sediment Se is 0.70. Those R^2 values which are not significant at $\alpha < .01$ are identified as N. S.

These variables also showed no significant correlation with water Se levels. However, sulfate, hardness, and conductivity did correlate well with each other. Table 8 summarizes the R^2 relationships between the water parameter variables as well as sediment Se level and organic sediment Se level. The R^2 values in Table 8 suggest that organic sediment Se levels are more strongly influenced by water Se level than by any other measured parameter. Since these two parameters tend to vary together, they would be expected to correlate in a similar fashion with organism Se levels. Table 7 shows this to be the case.

When the significant R^2 value in Table 7 for water Se and various organism Se levels are compared, all but three have a value greater than 0.90. The organisms with R^2 value below this level are damsel fly nymphs, plankton, and filamentous algae. This group shares an important feature in common. Each represents a genus mix. In the case of damsel flies (coenagrionidae), at least three genera are involved (see Table 6) and the plankton and algae probably represent an even more diverse array. In the other cases, with the probable exception of fish fry, the organism categories involved represent a single genus. The relationships between water Se levels and the Se levels in each of the 12 organism types analyzed as well as organic sediment are portrayed graphically in Figures 3 through 15. Linear regression equations of the general form, $y = a + bx$ are also given in these figures.

Table 8. Water Parameters and Sediment Coefficients of Determination (R^2)[1]

	Water Se	Organic Sediment Se	Sediment Se	Sulfate	Hardness
Water Se					
Organic Sediment Se	0.85				
Sediment Se	0.63	0.80			
Sulfate	N. S.	N. S.	N. S.		
Hardness	N. S.	N. S.	N. S.	0.93	
Conductivity	N. S.	N. S.	N. S.	0.97	0.85

[1] Those R^2 values which are not significant at $\alpha < .01$ are identified as N. S.

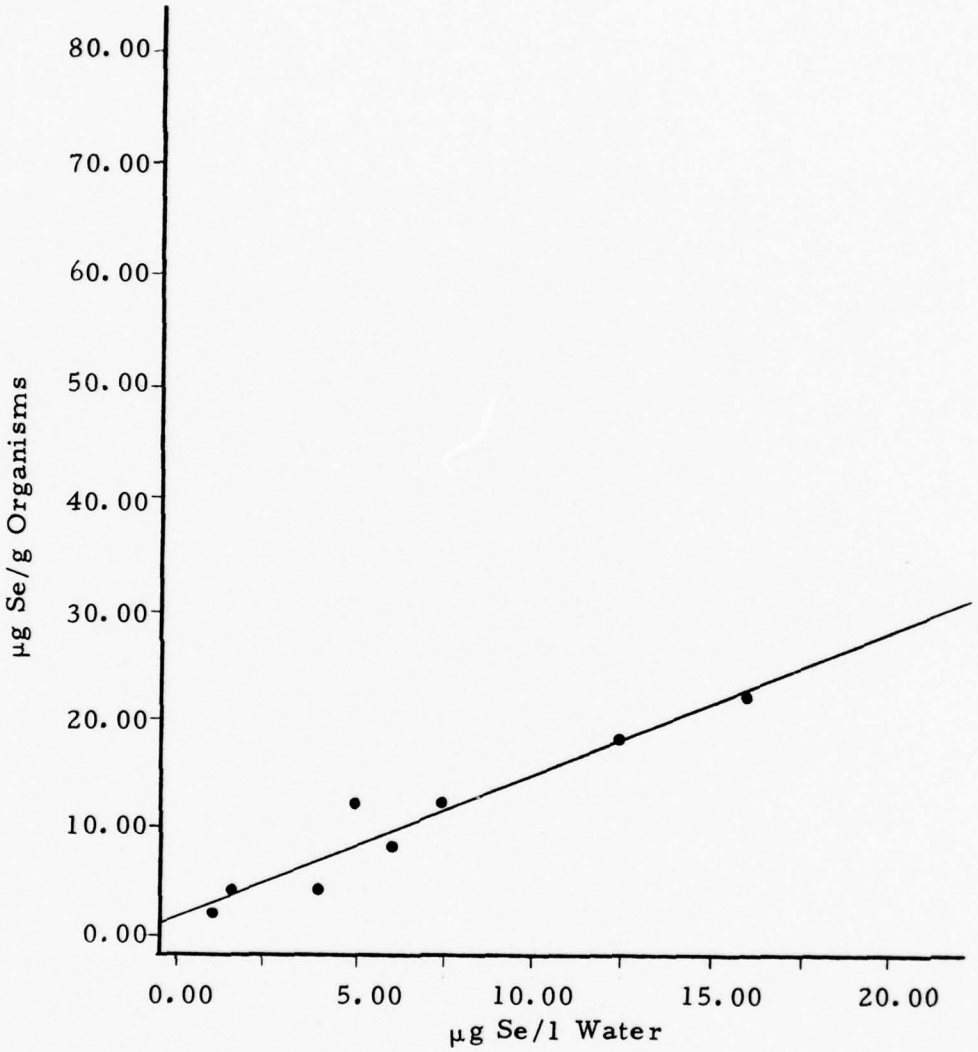

Figure 3. Hyalella azteca selenium levels.

Linear Regression Curve Equation: µg Se/g organism = 1.5 + 1.3 (µg Se/l water)
Bioconcentration Factor: 1300

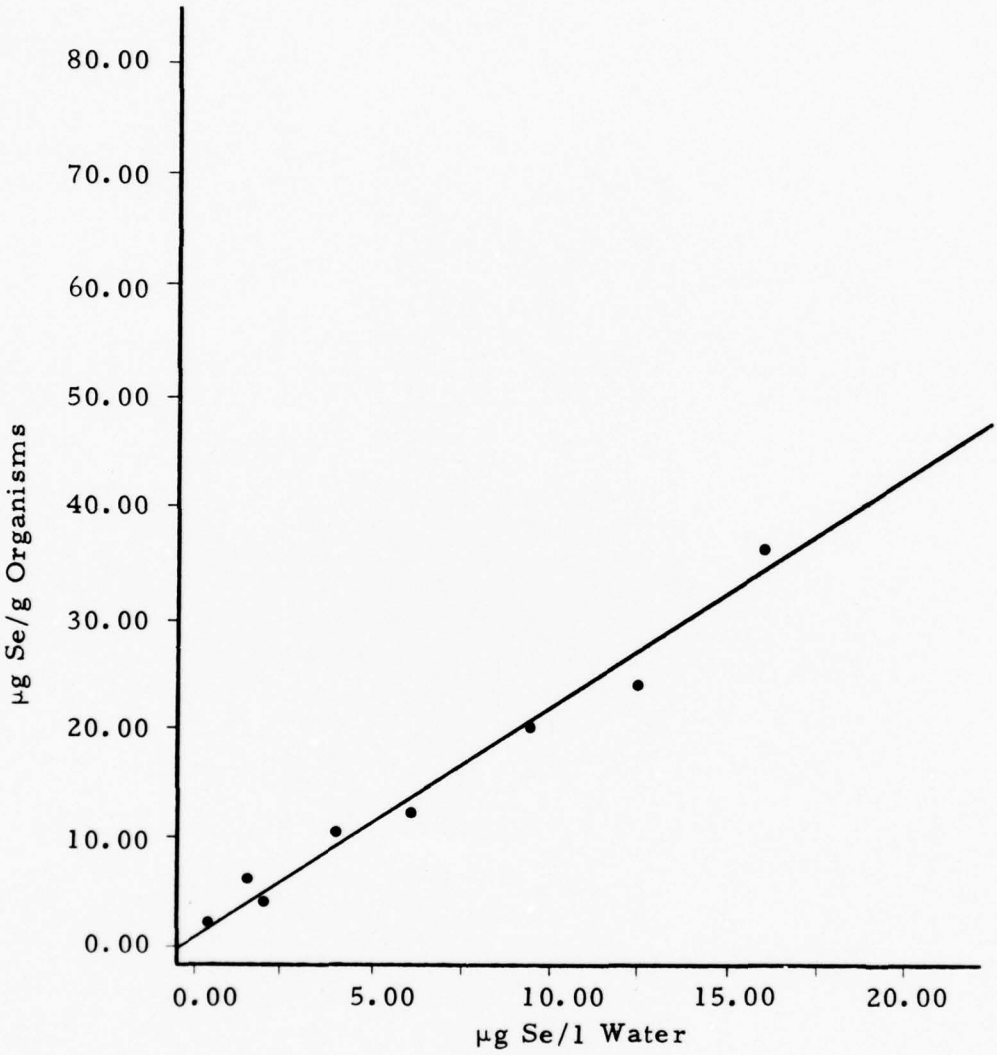

Figure 4. Astacidae Spp. selenium levels.

Linear Regression Curve Equation: µg Se/g organism = 0.4 + 2.1 (µg Se/l water)
Bioconcentration Factor: 2100

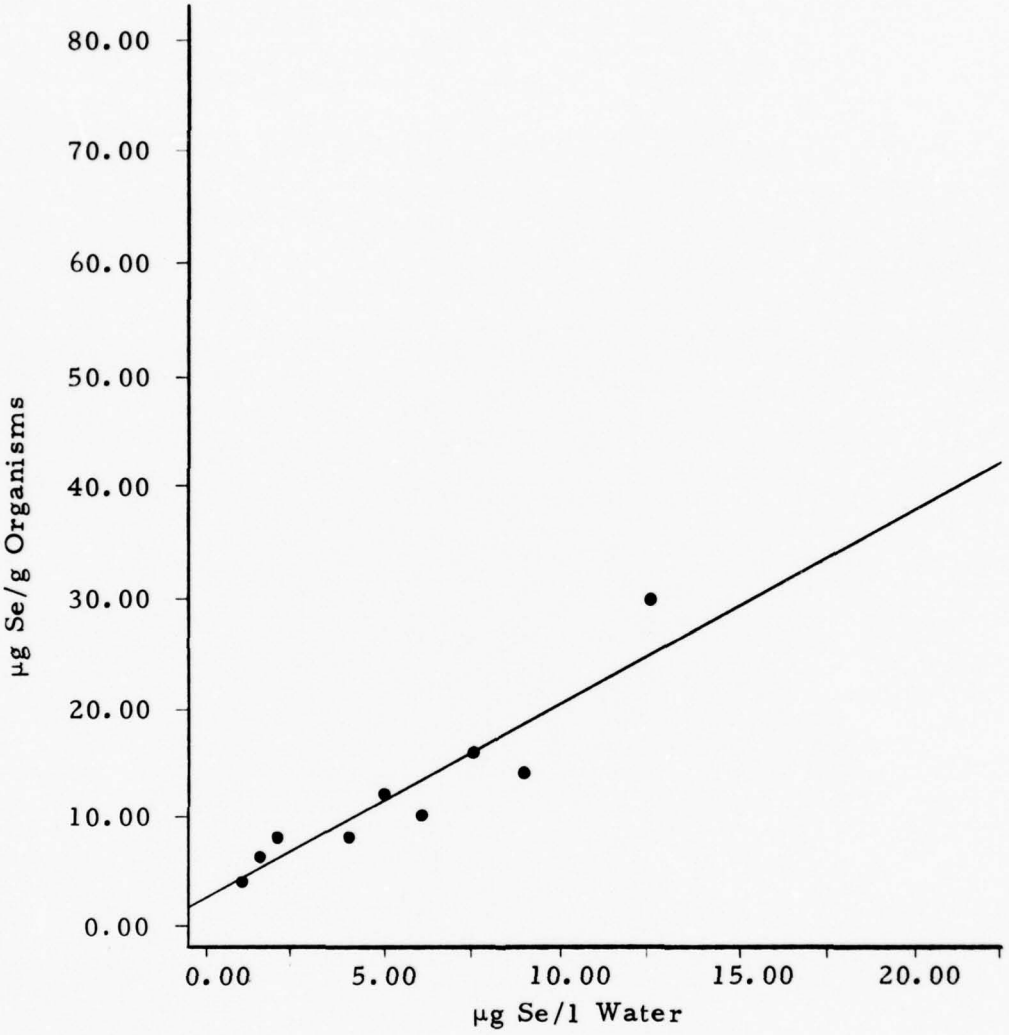

Figure 5. Cenocorixa Sp. selenium levels.

Linear Regression Curve Equation: µg Se/g Organism = 2.2 + 1.8 (µg Se/l water)
Bioconcentration Factor: 1800

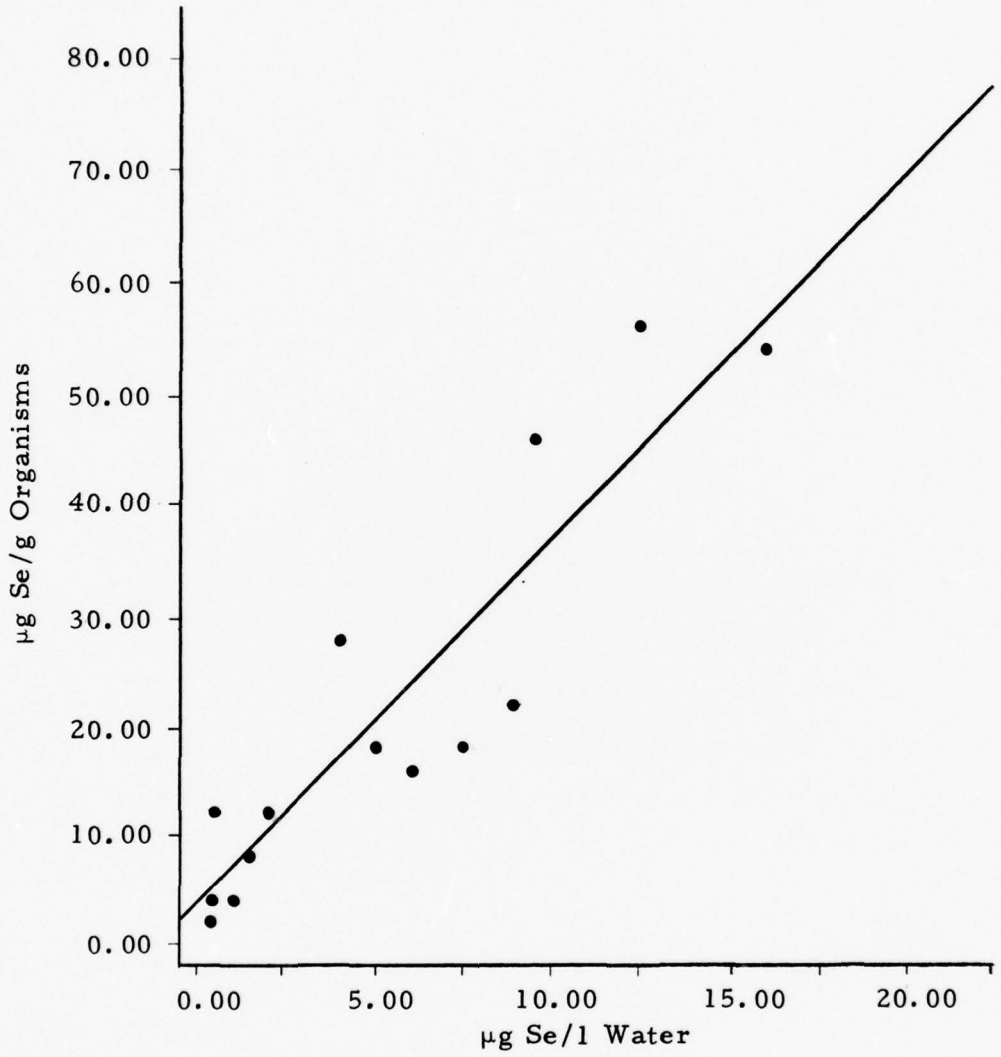

Figure 6. Coenagrionidae Spp. selenium levels.

Linear Regression Curve Equation: µg Se/g organism = 3.1 + 3.3 (µg Se/l water)
Bioconcentration Factor: 3300

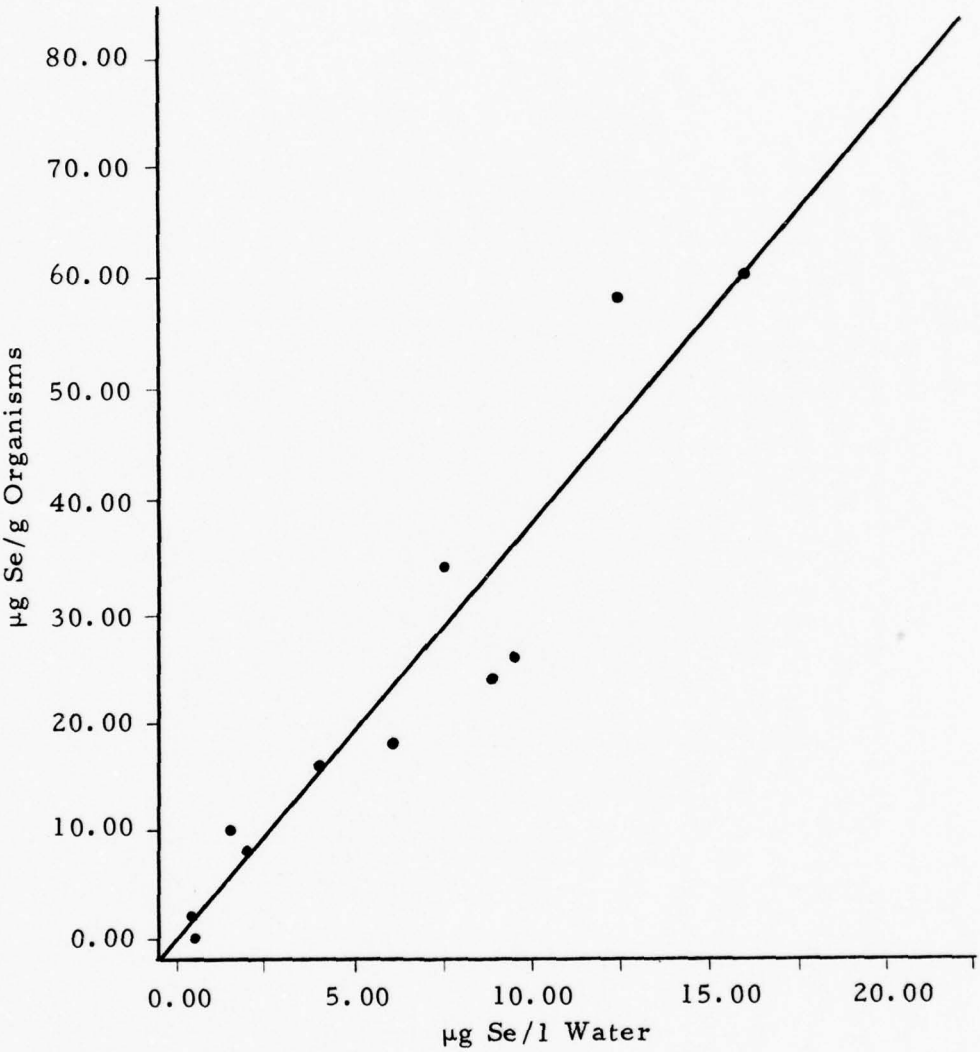

Figure 7. Chironomus Sp. selenium levels.

Linear Regression Curve Equation: µg Se/g organism = -0.6 + 3.8 (µg Se/l water)
Bioconcentration Factor: 3800

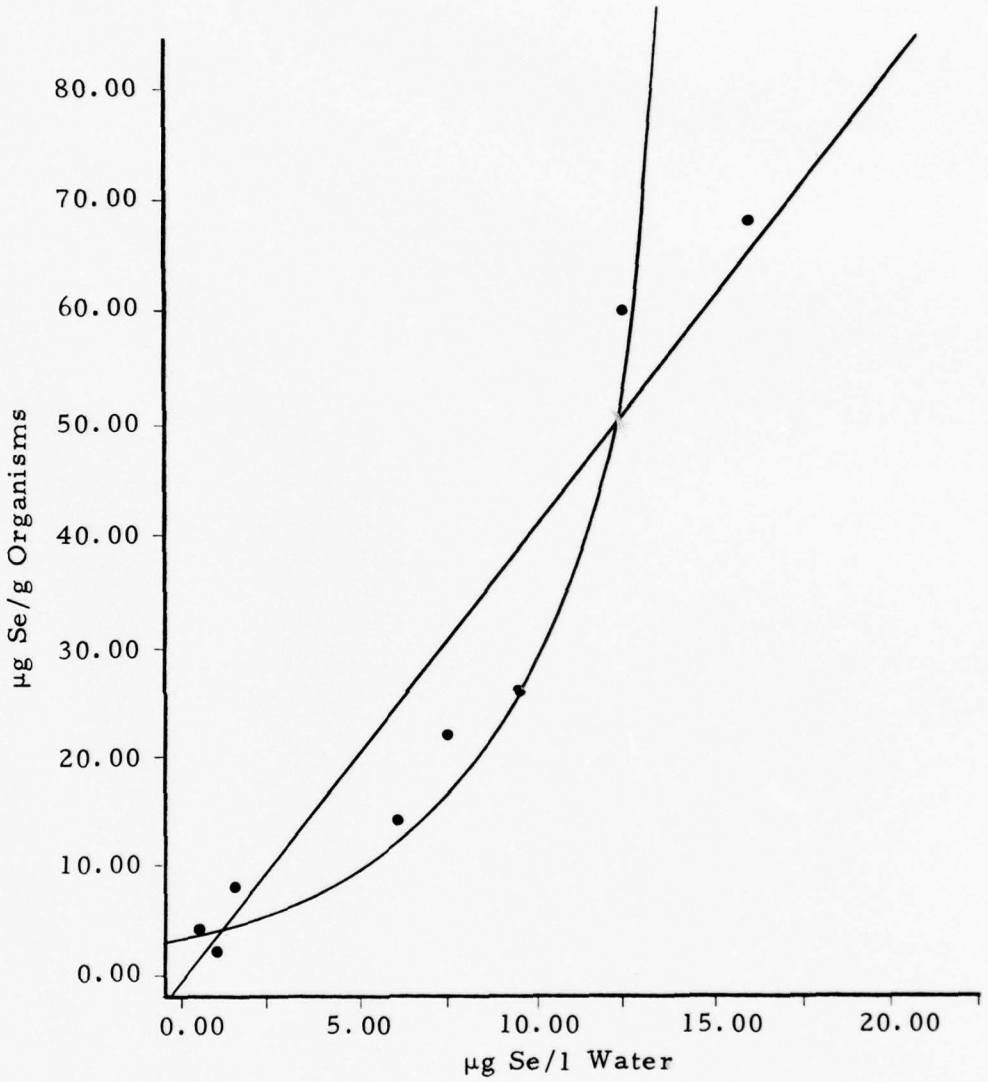

Figure 8. Fish fry selenium levels.

Linear Regression Curve Equation: µg Se/g organism = -2.7 + 4.2 (µg Se/l water)

Log Transform Regression Curve Equation: µg Se/g organism - $e^{[1.1 + 0.23 (\mu g\ Se/l\ water)]}$

Bioconcentration Factor: 4200[1]

[1] Based on nontransformed Linear Regression Equation

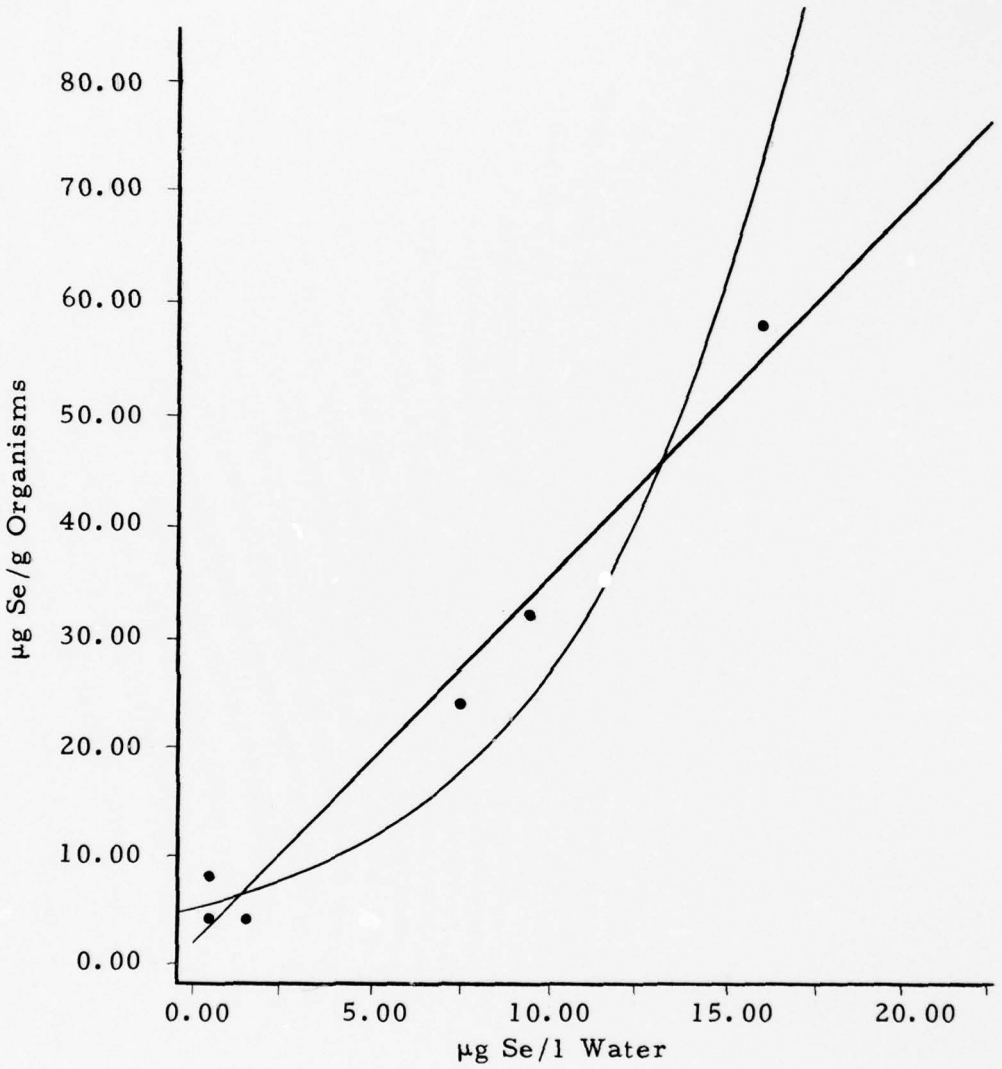

Figure 9. Fundulus kansae selenium levels.

Linear Regression Curve Equation: µg Se/g organism = 1.8 + 3.3 (µg Se/l water)

Log Transform Linear Regression Curve Equation: µg Se/g organism = $e^{[1.6 + 0.17 (\mu g\ Se/l\ water)]}$

Bioconcentration Factor: 3300[1]

[1] Based on nontransformed Linear Regression Equation.

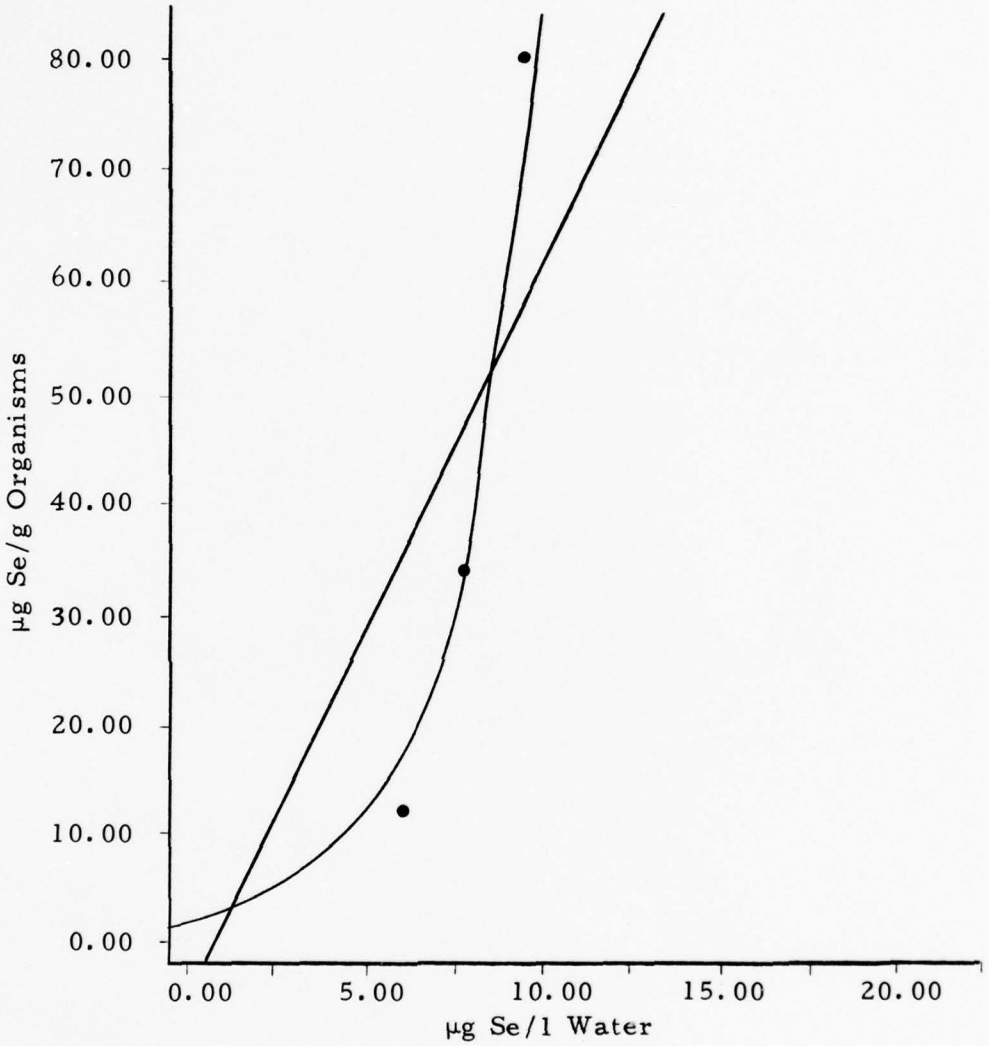

Figure 10. Pimephales promelas selenium levels.

Linear Regression Curve Equation: µg Se/g organism = -5.2 + 6.6 (µg Se/l water)[1]

Log Transform Linear Regression Curve Equation: µg Se/g organism = $e^{[0.53 + 0.39 (\mu g\ Se/l\ water)]}$

Bioconcentration Factor: < 8000[2]

---

[1] Coefficient of Determination (R^2) not significant at $\alpha < .01$.

[2] Based on observed data and not on regression analysis.

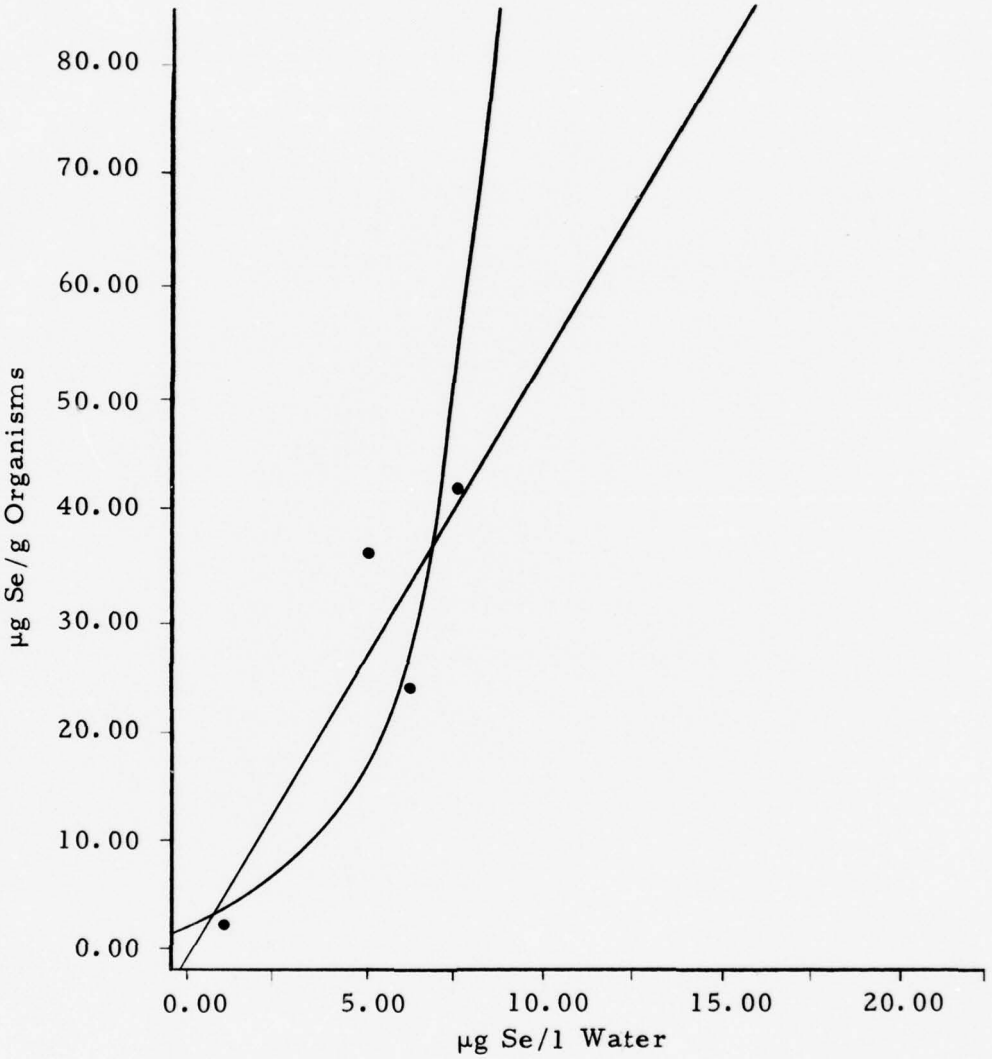

Figure 11. Etheostoma exile selenium levels.

Linear Regression Curve Equation: µg Se/g organism = -0.9 + 5.4 (µg Se/l water)[1]

Log Transform Linear Regression Curve Equation: µg Se/g organism = $e^{[0.66 + 0.45 (\mu g\ Se/l\ water)]}$[1]

Bioconcentration Factor: < 8700[2]

---

[1] Coefficient of Determination (R^2) not significant at $\alpha < .01$.

[2] Based on observed data and not on regression analysis.

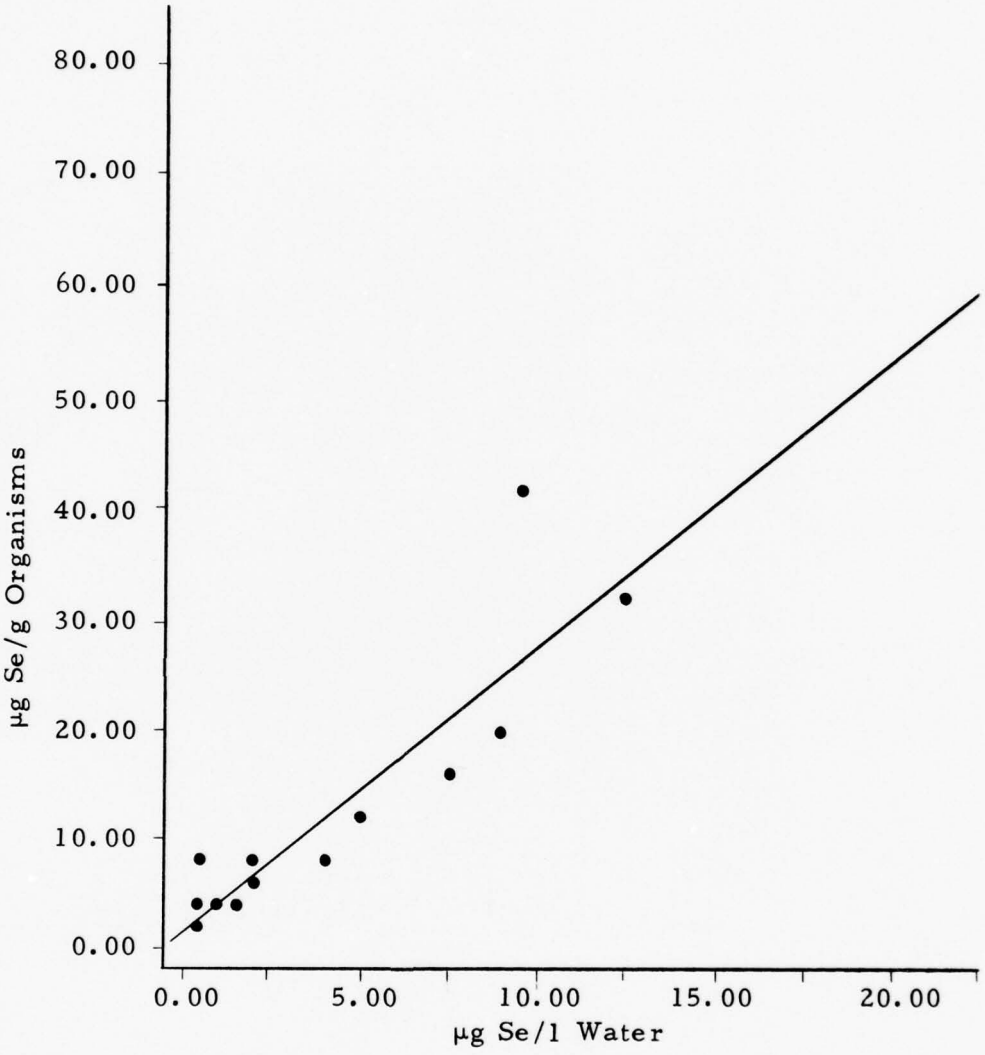

Figure 12. Plankton selenium levels.

Linear Regression Curve Equation: µg Se/g plankton = 1.3 + 2.6 (µg Se/l water)
Bioconcentration Factor: 2600

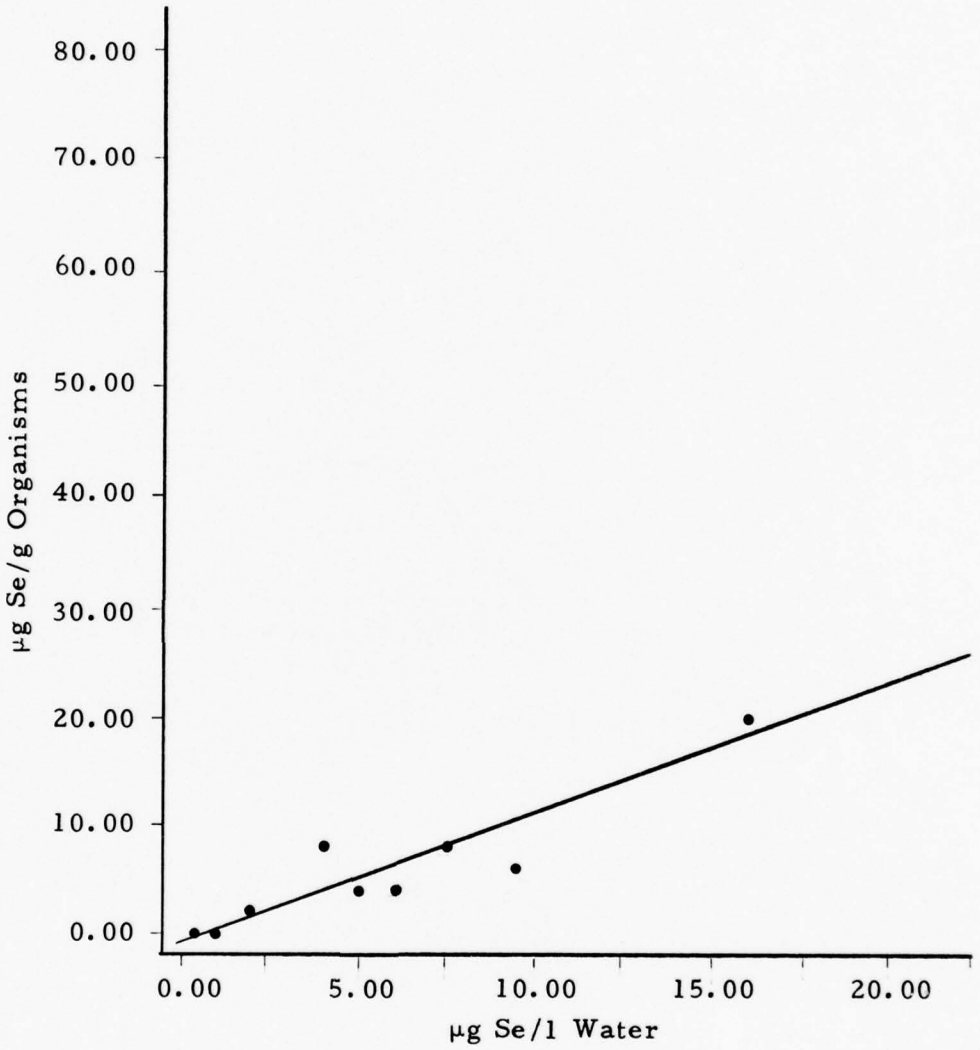

Figure 13. Filamentous algae selenium levels.

Linear Regression Curve Equation: µg Se/g organism = 0.8 + 1.2 (µg Se/l water)
Bioconcentration Factor: 1200

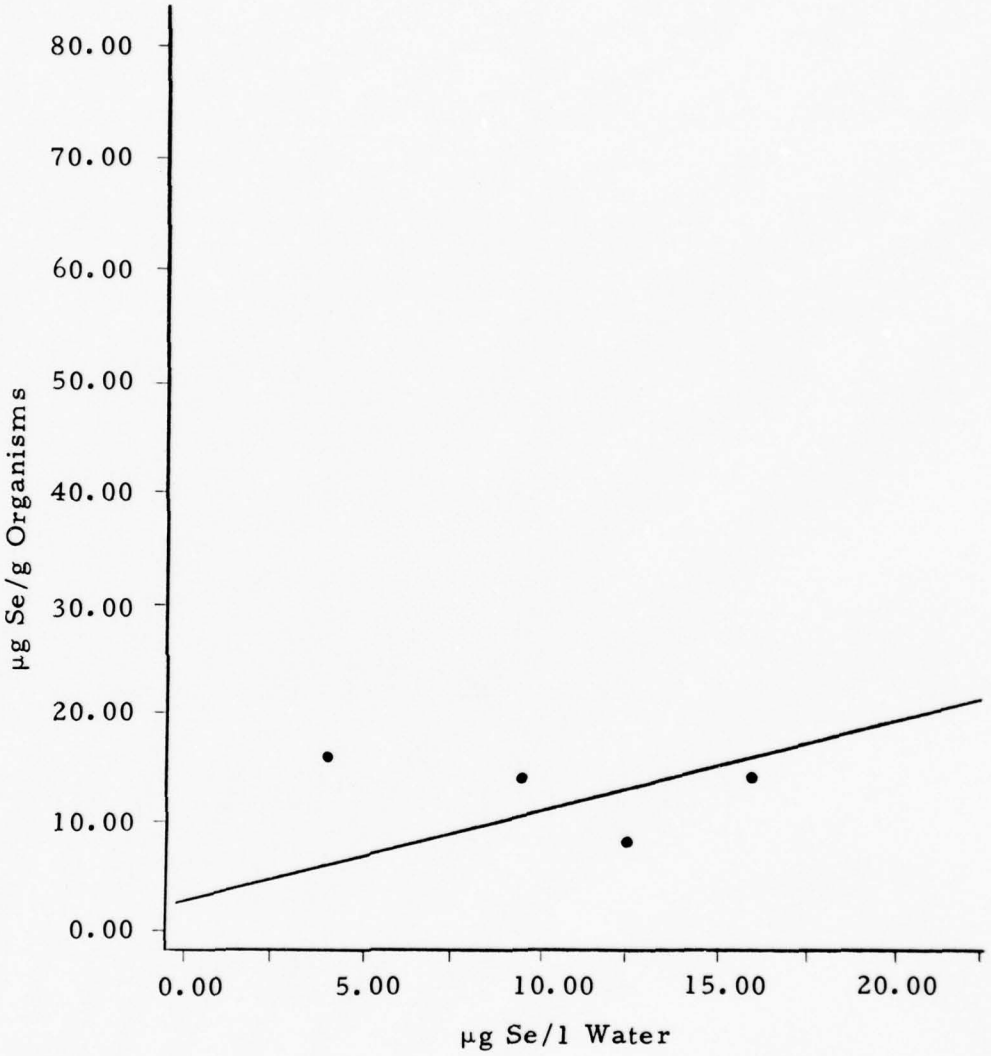

Figure 14. Chara Sp. selenium levels.

Linear Regression Curve Equation: µg Se/g organism = 2.7 + 0.8 (µg Se/l water)[1]
Bioconcentration Factor: < 4200[2]

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[1] Coefficient of Determination (R^2) not significant at $\alpha < .01$.

[2] Based on observed data and not on regression analysis.

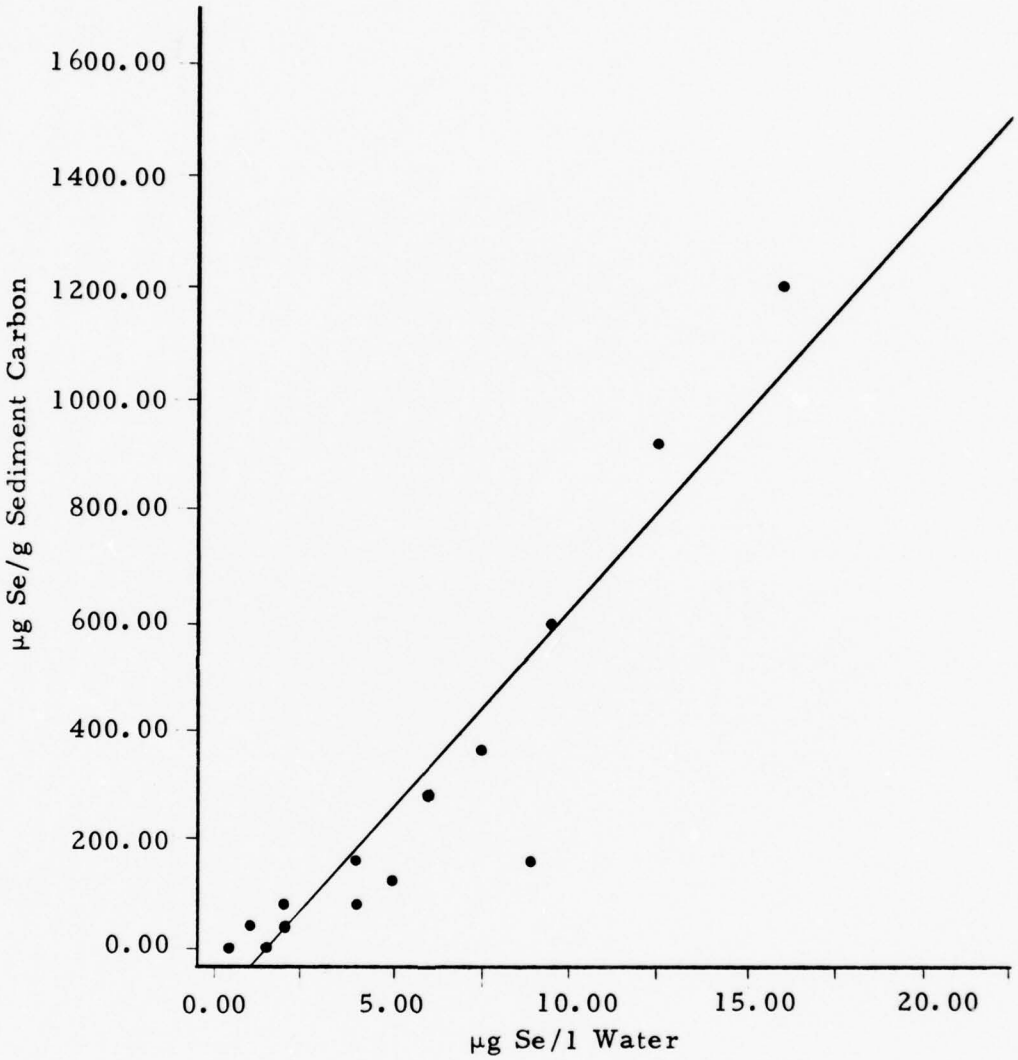

Figure 15. Organic sediment carbon selenium levels.

Linear Regression Curve Equation: µg Se/g sediment carbon = -130.0 + 73.0 (µg Se/l water)
Bioconcentration Factor = 73,000

The biological significance of the terms in these equations are interpreted as follows:

Variable y --organism Se level (μgSe/g)

Intercept (a)--Minimum organism Se level (μgSe/g)

Slope (b)--Bioconcentration Factor/1000

Variable X--Water Se level (μgSe/1)

It should be noted that if Se is not required by the organisms, the value of the intercept (a) could theoretically be zero. If Se is a required constituent of the organism's chemical makeup, then this value could not be zero even if the element were not detectable elsewhere in the organism's habitat. An intercept (a) with a negative value has no meaning in the biological context and suggests the possibility of measurement error and/or a nonlinear relationship between tissue and water Se levels (nonlinear regression).

The use of the term "bioconcentration factor" is not meant to imply that the particular organism to which it applies actually concentrated the Se from water. Instead, it is a measure of the apparent difference between tissue level regardless of what may have caused the difference to occur. The relationship between the slope (b) and bioconcentration factor involves a factor of 1000 because the organism level units expression (μg Se/g) is 1000 times greater than the water level expression (μg Se/1). Thus, the bioconcentration factor for organisms included in this study can be obtained by multiplying the slope of the calculated regression line for that organism by 1000.

The more accurately the slope value is known, the more reliable the bioconcentration factor becomes.

In most of the cases involving fish, the curves exhibited a possible exponential character and most of the intercepts are negative. Therefore, the data was transformed using natural logarithm (ln or $\log_e$). This is based on the assumption that the relationship between water Se (x) and organism Se (y) might be exponential and explained by an equation of the form, $y = e^{a+bx}$. The expression, $y = e^{a+bx}$ is equivalent to $y = Ae^{bx}$ where $A = e^a$. The logarithmic transform puts the relationship into the form, $\ln y = a+bx$ which can be subjected to linear regression analysis. The R^2 values for the transformed variables are given in Table 9. In the case of fish fry, similar R^2 values suggest that the exponential and linear curves fit the data equally well. For F. kansae, the exponential fit yields a lower R^2 value (poorer fit). For P. promelas, the exponential fit yields a much improved R^2 value (better fit). The small number of scattered data points available for E. exile do not yield a statistically meaningful exponential or linear fit. However, the graphed data for this organism suggest some kind of dependency between tissue and water Se levels. Overall, the exponential fit does have the advantage of avoiding the biologically meaningless negative intercept in those cases where it occurred. The exponential curves derived from a linear fit of the transformed data are portrayed in Figures 8 through

Table 9. Logarithmic Transform[1] Fish Selenium Level Coefficients of Determination $(R^2)^2$

Organism	Water Se	Organic Sediment Se	Sediment Se
Fish Fry	0.90	0.78	N. S.
Eundulus kansae	0.91	N. S.	N. S.
Pimephales promelas	0.98	N. S.	N. S.
Etheostoma exile	N. S.	N. S.	N. S.

[1] Least Squares correlation-regression was performed using the natural logarithm (ln) of the Fish Se levels (y). The intercept (a) and the slobe (b) of the resulting regression line ($\ln y = a + bx$) was used to form an exponential fit curve according to the equation $y = e^{a+bx}$ where x is the water Se level (see Figures 8 to 11).

[2] Those R^2 values which are not significant $\alpha < .01$ are identified as N. S.

11. Of all the organisms studied, Chara (a "rooted" alga) exhibited Se levels that seemed the least dependent on water Se level (see Figure 15).

In summary, the correlation and regression analysis of the whole body organism and organic sediment Se levels revealed a very strong dependency on the prevailing water Se level in the habitat and was not influenced to any measurable degree by other water parameters. For some of the fish, the possibility of an exponential relationship exists. However, for most of the other organisms studied, a linear fit is adequate to explain the data. Regression curves and bioconcentration factors based on regression equations for the five organisms exhibiting the highest R^2 values when compared to water Se levels are portrayed in Figure 16. The relationships between organism and organic sediment Se levels are quite similar to those just described for organism and water levels of the element (see Table 7). For purposes of comparison, Figure 17 portrays the regression curves obtained for organic sediment and the five organisms included in Figure 16.

Tissues from adult fish (muscle and liver) netted at three different study sites were also analyzed for Se. The results are given in Table 10. Four generalizations are apparent in these data. First, tissue Se levels are quite variable from fish to fish. This could be due to the fact that the fish are not all indigenous to the site where caught or to the fact that there is considerable inherent variability

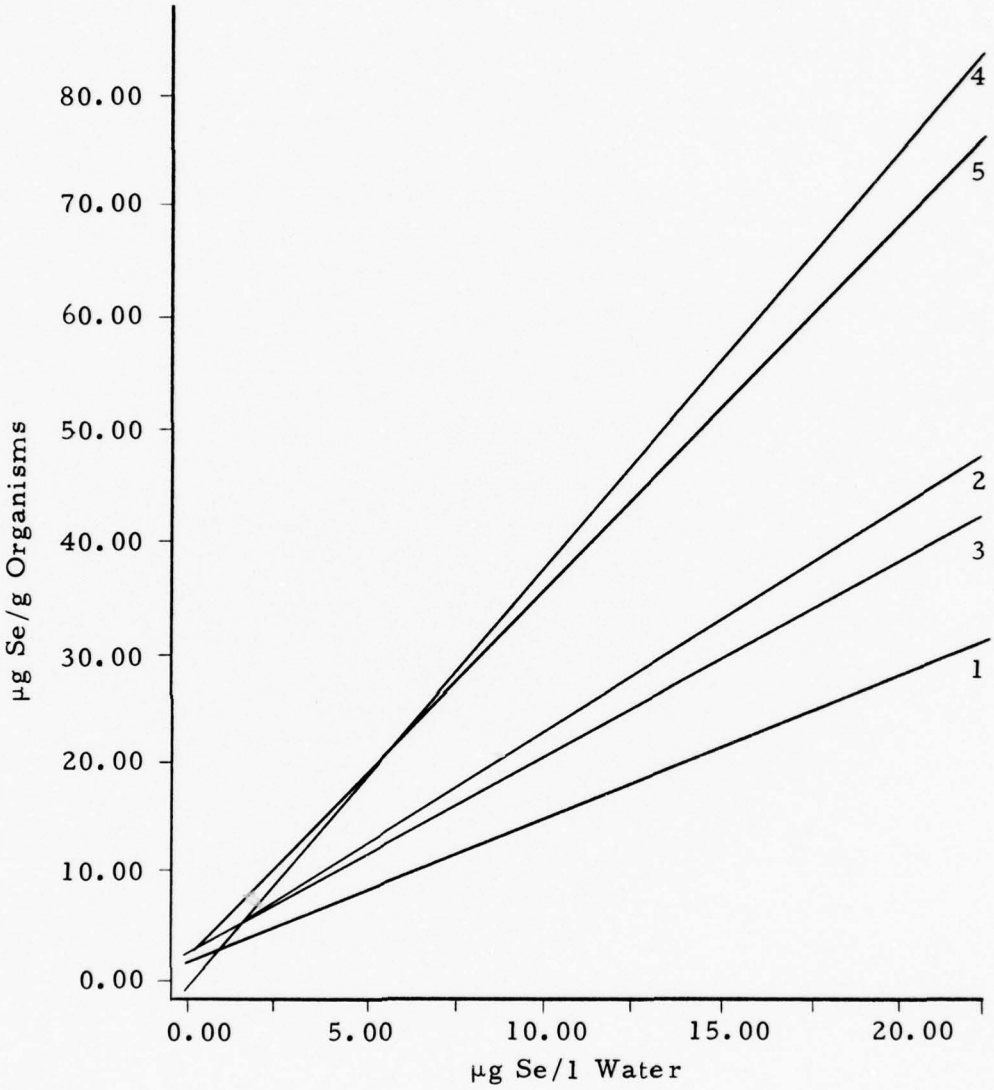

Figure 16. Organism and water selenium level prediction curves.[1]

Curve	Organism	Bioconcentration Factor
1	Hyalella azteca	1300
2	Astacidae Spp.	2100
3	Cenocorixa Sp.	1800
4	Chironomus Sp.	3800
5	Fundulus kansae	3300

[1] Based on Linear Regression Equations having coefficients of determination (R^2) all having values greater than 0.90.

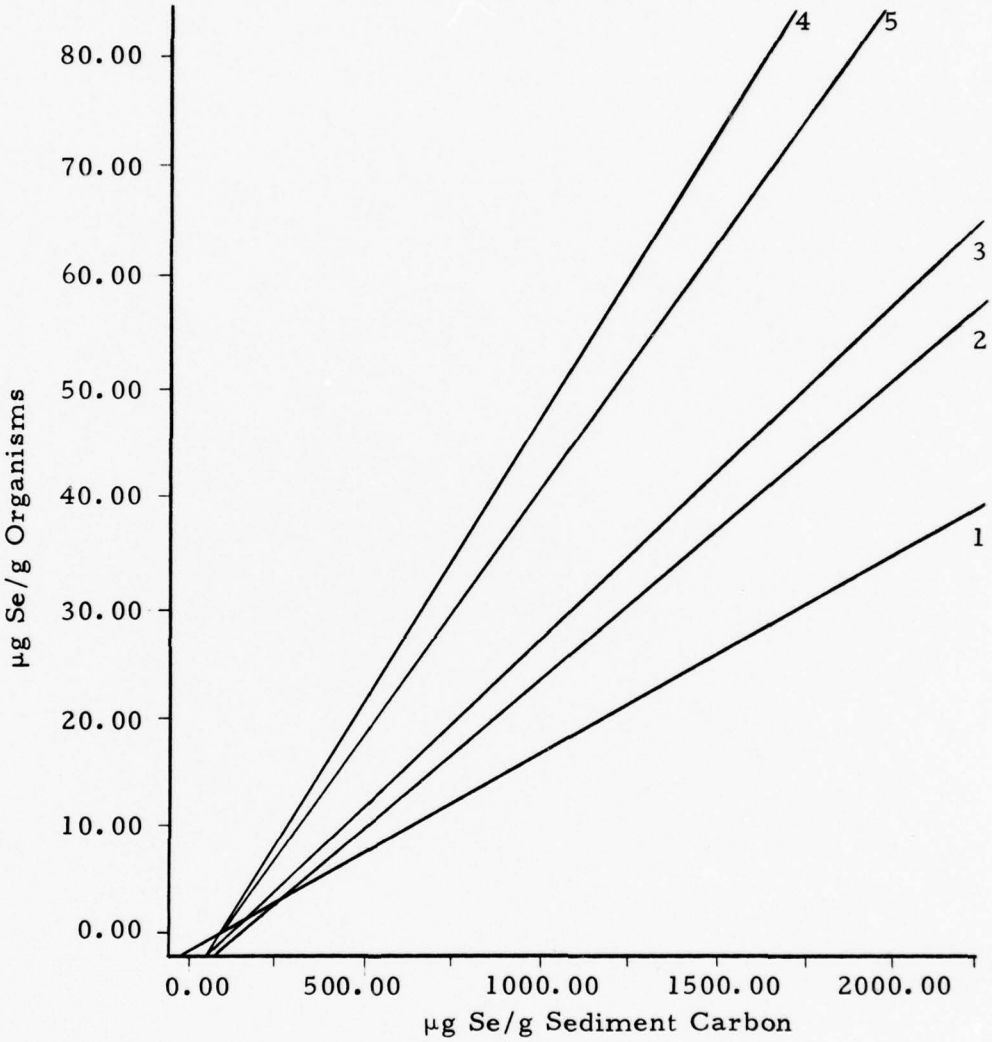

Figure 17. Organism and organic sediment selenium level prediction curves.[1]

Curve	Organism
1	Hyalella azteca
2	Astacidae Spp.
3	Cenocorixa Sp.
4	Chironomus Sp.
5	Fundulus kansae

[1] Based on Linear Regression Equations having coefficients of determination (R^2) all having values greater than 0.89.

Table 10. Fish Muscle and Liver Tissue Selenium Levels

	Locations and Mean Water Se Levels								
	Mac Mesa Reservoir 2.2 µg/liter			Highline Reservoir 4.2 µg/liter			Sweitzer Lake 9.4 µg/liter		
	µg/g Se		(cm)	µg/g Se		(cm)	µg/g Se		(cm)
	Muscle	Liver		Muscle	Liver		Muscle	Liver	
Lepomis cyanellus Green sunfish	14.6	8.3	(9)	25.8	30.5	(15)	93.6	126.7	(8-22)[1]
	25.8	25.9	(15)	46.7	50.4	(12-16)[1]	110.2	97.1	(21-22)[1]
means	20.2	17.1		36.3	40.5		101.9	111.9	
Ictalurus melas Black bullhead				31.8	51.1	(18)	47.8	105.1	(20)[1]
							54.1	72.2	(29)
							39.0	62.6	(29)
means				31.8	51.1		50.1	80.0	
Gila sp. Chub				29.9		(35)	85.3		(21-25)[1]
							82.3		(40)[1]
means				29.9			83.8		
Cyprinus carpio Carp				36.3		(16)	85.4		(23)
				61.8		(47)	72.5		(33)
means				49.1			79.0		
Catostomus commersoni White Sucker				77.8		(40)[1]	85.9		23[1]
							104.0		(30-34)[1]
means				77.8			95.0		
Catostomus catostomus Longnose sucker				59.0		(30)[1]	99.0		(43)[1]
Catastomus latipinnis Flannel mouth sucker				61.3	77.9	(44)			
				15.9	73.1	(49)			
				20.0	76.7				
means				32.4	75.9	(53)			
Ictalurus punctatus Channel Catfish							56.8	77.0	(30)[1]
							57.6	66.7	(45)
							76.6	78.1	(45)
means							63.7	73.9	

[1] Analyzed sample was a composite of tissue from two or more fish.

from individual to individual. Second, the mean Se levels tend to be higher in those fish from habitats with higher water Se levels. This is consistent with the results obtained from the whole body analysis of smaller fish described earlier. Third, the liver tissue Se levels tend to be higher than those in muscle. This is consistent with the results obtained by others for both terrestrial and aquatic organisms (see Tables 1 and 2 and Appendix 4). Finally, muscle and liver levels tend to be higher than those observed for whole body analysis of fish from those same study sites (see Table 6). This is consistent with the fact that other tissues such as bone are included in the whole body analysis and these tissues tend to have lower levels of Se than do muscle and liver.

Crayfish (Astacidae) tissue levels for muscle, gill, and carapace from three locations were also compared and the results are given in Table 11. Two trends are apparent. For any one tissue type, a clear dependency on habitat Se levels is apparent. This was also the case for the whole crayfish analysis described earlier. When tissues are compared, muscle has a slightly higher concentration than gills and both of these tissues exhibit much higher levels than carapace.

The low levels of Se for carapace tissue suggests that those anthropods having a higher proportion of exoskeleton relative to total body mass might be expected to exhibit lower whole body Se levels. It is interesting to note that the organisms having the two lowest Se

Table 11. Crayfish Tissue Selenium Levels[1]

Location	Park Creek Reservoir	Highline Reservoir	Desert Reservoir
Water Se Level	0.3 µg/liter	4.2 µg/liter	15.8 µg/liter
Number of Organisms	2	2	2
Muscle µgSe/g	1.2	21.5	54.1
Gills µgSe/g	--	14.2	45.9
Carapace µg/Se/g	0.5	0.7	7.0

[1] Organisms were Astacidae Spp., 6 to 12 cm long. Values given are the means of duplicate analysis of combined tissues from 2 or more organisms.

levels (Hyalella azteca and Cenocorixa) have relatively thick exoskeletons. Those species having the higher Se levels are chronomid larvae and damsel fly nymphs which have thin, delicate exoskeletons. One source of variability in the results obtained in whole body analysis for Se for a given species from a given habitat could relate the exoskeleton development. In organisms that have just molted, the Se level might be higher due to a lower proportion of exoskeleton than for those individuals with older and presumably heavier exoskeletons.

The data discussed thus far were collected for the purpose of comparing Se levels as they vary from habitat to habitat. Reliable conclusions regarding variations in Se level for a particular organism in its habitat over time are not generally derivable from this data. The one exception to this is based on sampling done in the fall of 1976 at Twin Buttes Reservoir (location given in Table 3). An indigenous population of rapidly growing F. kansae fry was sampled at that site seven times over a 99 day period. The analytical results are portrayed in Table 12. For the period sampled, there is no indication that whole body Se levels per unit weight increased although body weight increased an average of nearly six-fold during that time. It should be noted that this result does not rule out such an increase over much longer time intervals. It also does not rule out the possibility of such increase with time in particular types of tissue such as liver.

This result is consistent with what has been reported concerning the excretory dynamics of the element. Se is a known constituent of

Table 12. Fundulus kansae Serial Sampling Selenium Levels[1]

Date	Mean Se Level µg/g	Mean Fish Weight g	Number of Fish	Number of Determinations[2]
29 Jul	21.9	0.0068	30	2
12 Aug	19.1	0.0106	26	2
26 Aug	21.4	0.0675	33	15
9 Sep	21.8	0.0520	14	7
7 Oct	21.3	0.0422	14	7
21 Oct	22.5	0.0352	23	10
4 Nov	19.2	0.0390	12	6

[1] Fish were netted from shallow schooling areas at Twin Buttes Reservoir in 1976.

[2] The number of determinations necessary to accommodate the number of fish available for whole body analysis.

mammalian urine. Se metabolites can be excreted and do not have to be retained indefinitely (see Appendix 1). The results of this study suggest that this may also be true for lower organisms residing in aquatic habitats.

The presumed ability of aquatic organisms to excrete Se metabolites raises another important question. Why is it that many of the organisms included in this study have such consistent apparent bioconcentration factors? When the organisms are confronted with a seleniferous habitat, why can they not avoid high levels of tissue Se by simply excreting the element? One possible explanation for this can be deduced on the basis of what has been reported about Se metabolism. An organism such as a fish derives many of its amino acids from the lower organisms it consumes. These in turn ultimately derive the amino acids from primary producers. There is evidence to suggest that Se is incorporated into amino acids as a sulfur analog during protein synthesis by photosynthetic organisms. Once incorporated into the molecular structure of plant amino acids, the Se atoms will eventually come to occupy a place in the protein structure of those organisms which derive their nutrition directly or indirectly from plants. This Se would not be released for possible excretion until the amino acids are broken down to yield catabolic products. Since the consumer organisms must conserve amino acids to some degree for protein synthesis rather than break them down, the Se will tend to be conserved as well. This phenomenon could be

expected to result in apparent bioconcentration factors for consumers that are similar to those of the producers which are probably responsible for actually concentrating the Se from the surrounding water.

Another possible explanation for why organisms from seleniferous habitats other than plants have high tissue levels of Se is that trophic level biomagnification of the element may be occurring. Biomagnification refers to the process in which successively higher levels of a substance are seen in the tissue of organisms in successively higher trophic levels of the food chain (Woodwell, 1967). The data obtained in this study reveal that in the food chain extremes represented by algae and fish liver, the largest observed biomagnification for Se is no greater than six-fold. This was derived from the levels in filamentous algae when compared to green sunfish liver at Sweitzer Lake (see Tables 6 and 10). Such a low degree of difference between organisms at markedly different levels of the food chain may not even represent biomagnification. Instead, it may be a reflection of the difference in biological composition between algae and liver. The effect of composition differences on Se level can be seen in Table 11. When tissue from the same organism are compared such as the carapace and muscle of an individual crayfish, Se differences even greater than six-fold are seen. Therefore, it is reasonable to expect such differences would also be observed when organisms of radically different biochemical make-up are compared. While the results of this study do not rule out the possibility of Se

trophic level biomagnification, the data do suggest that its contribution to the tissue levels observed is minor. The differences that are observed when different organism types are compared can be understood in terms of other factors such as tissue composition difference and the conservative nature of amino acid metabolism.

In closing this discussion of the field study results, it is important to emphasize that the "linearity" of the relationship between water and organism levels of Se has been demonstrated for only a portion of the range over which Se levels could conceivably vary in aquatic habitats. Field or experimental work designed to provide data on the effects of Se levels in water not included in this study (below 0.3 μg/l or above 15 μg/l) could well reveal a departure from a linear relationship. The exponential appearance of the data for fish suggests that at very low water Se levels, apparent bioconcentration factors may tend to be higher. It may also be that the factors would be shown to diminish at very high water Se levels. Overall, such a pattern would produce a logistic or "S" shaped curve for the relationship between organism and water Se levels. The curve would exhibit an initial increase in slope, a linear portion, and then a decreasing slope. If actually found to exist, such a curve could be interpreted as showing the ability on the part of aquatic consumers and their prey organisms to conserve Se when it is scarce and discard Se when it is in excess. Clearly, it is the upper portion of the curve that is in need of better definition if one is especially interested in the dynamics

of Se as a "pollutant." As for the lower portion of the curve, it could provide information about the value of investigating Se as a possible "limiting factor" for aquatic community production when the element is present at very low levels.

Experimental Results

The aquaria studies of Se effects were not entirely successful in that mortality was excessive in three of the 15 aquaria. However, analysis of those fish which survived to the end of the experimental procedure revealed a definite pattern of tissue Se response in both experiments.

The results of the experiments are tabulated in Tables 13 and 14 and illustrated graphically in Figures 18 and 19. By inspection, those differences in tissue Se levels that are observed seem attributable to food and not to water. This apparent result was verified by tests of statistical significance using one-way analysis of variance (ANOV) techniques. It should be noted that while the experimental design does lend itself to analysis by two-way ANOV, this approach is not considered appropriate for situations where sample sizes are unequal or where there is missing data. Both circumstances apply in this case.

The results from both experiments were subjected to two ANOV procedures. First, the variability in tissue Se level for each fish associated with each of the different food and water combinations

Table 13. Pimephales promelas Selenium Uptake Experiment (3x3 Array)

Estimated initial mean weight of fish[1]					0.0120 g					
Final mean weight of experimental fish					0.0507 g					
Estimated initial mean Se level of fish[1]					13.9 µg/g					
Tank Number		1	2[2]	3	4	5	6	7[2]	8	9
Surviving Fish		9	0	9	4	9	9	1	8	7
Experimental Parameters	µgSe/l water	3.1	4.4	7.5	3.2	5.3	8.3	3.9	6.0	8.8
	µgSe/g food	1.2	1.2	1.2	5.7	5.7	5.7	11.8	11.8	11.8
Final Tissue Level µgSe/g	Range	2.2-12.7	--	2.5-8.1	4.8-9.4	2.9-5.8	4.6-6.1	9.0	6.5-16.3	5.6-18.1
	Standard Deviation	3.0	--	2.0	1.9	1.8	0.9	--	2.8	4.1
	Mean	5.7	--	5.0	7.0	5.2	5.4	--	11.0	10.3

Analysis of Variance Table			
Source	Degrees of Freedom	Sum of Squares	Mean Squares
Total	54	599.5	
Treatment	6	311.4	51.9
Error	48	288.1	6.0
Calculated F-value			F = 8.6
Table F-value		F .05(6, 48)	= 2.3
Comparison of F-values indicates that there is a significant difference between Fish Se levels.			

Student-Neuman-Keals Comparison of Means						
Tank Number	5	6	1	4	9	8
Means	5.2	5.4	5.7	7.0	10.3	11.4
Means are arranged in order of increasing magnitude. Those means not sharing common underlining are considered significantly different at $\alpha < .05$.						

[1] Weight and Selenium level given are those of fish netted at Miller's Lake with the experimental fish and judged equivalent to the experimental fish.

[2] Omitted from the statistical analysis.

Table 14. Fundulus kansae Selenium Uptake Experiment (2x3 Array)

Experimental Results							
Initial mean weight of fish[1]			0.0065 g				
Final mean weight of fish			0.0261 g				
Initial mean selenium level of fish[1]			22.5 μg/g				
Tank Number		1	2	3	4	5[2]	6
Surviving Fish		9	8	10	5	1	9
Experimental Parameters	gSe/l Water	3.3	5.1	8.1	3.7	5.4	8.9
	gSe/g Food	5.7	5.7	5.7	11.8	11.8	11.8
Final Tissue Level gSe/g	Range	5.4-12.8	7.3-11.2	7.6-11.1	11.9-18.6	19.2	10.2-16.8
	Standard Deviation	2.6	1.4	1.1	3.1	--	2.3
	Mean	8.4	8.5	9.2	15.1	--	14.5

Analysis of Variance Table			
Source	Degrees of Freedom	Sum of Squares	Mean Square
Total	40	49.4.7	
Treatment	4	333.7	83.2
Error	36	162.1	4.5
Calculated F-value		F =	18.5
Table F-value		F .05(4136) =	2.6
Comparison of F-values indicates that there is a significant difference between fish Se levels			

Student-Neuman-Keals Comparison of Means						
Tank Number		1	2	3	6	4
Means		8.4	8.5	9.2	14.5	15.1
Means are arranged in order of increasing magnitude. Those means not sharing common underlining are considered significantly different at $\alpha < .05$.						

[1] Weight and Selenium levels given are those of fish netted at Twin Buttes Reservoir with the experimental fish and judged equivalent to the experimental fish.

[2] Omitted from the statistical analysis.

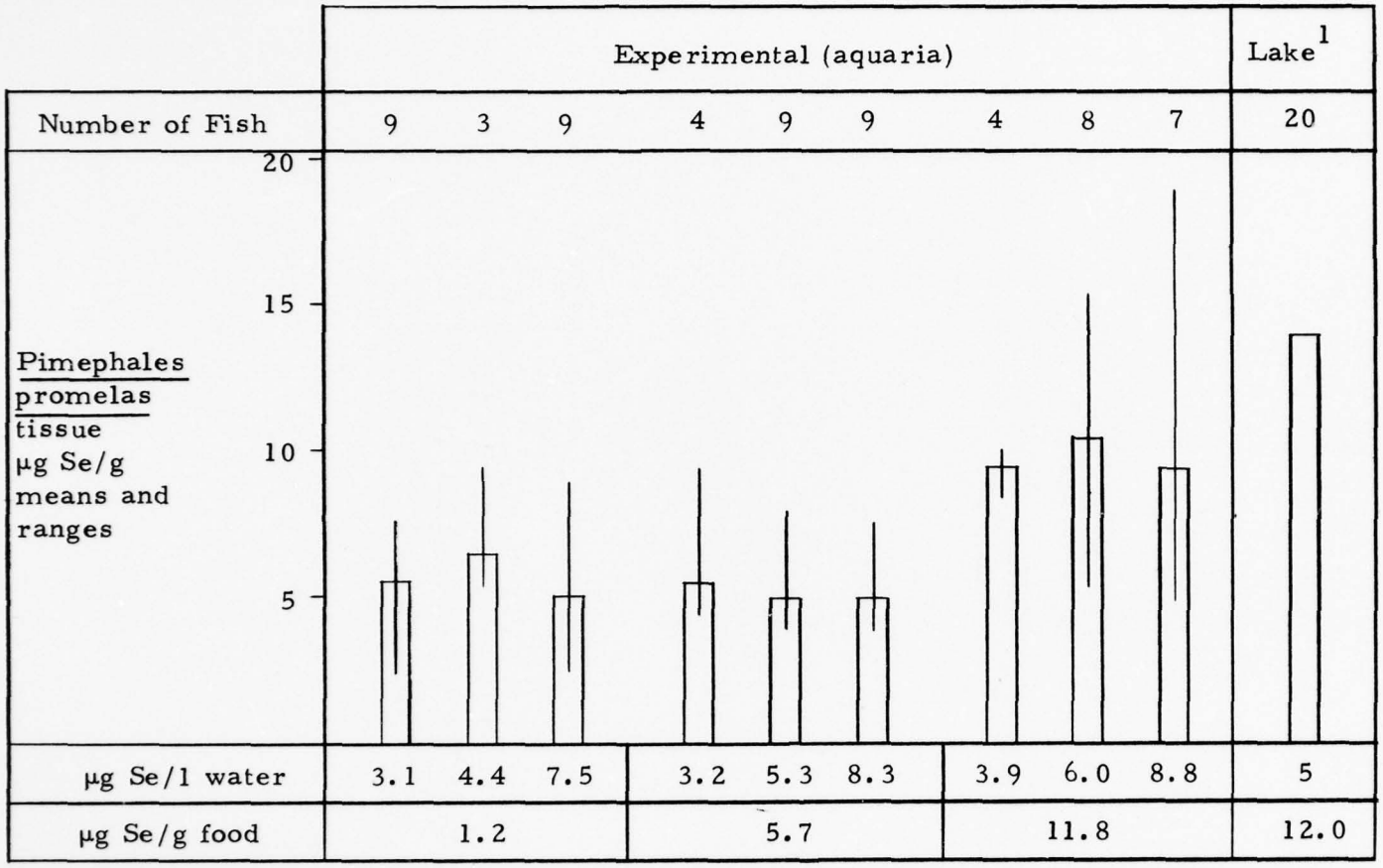

Figure 18. Selenium uptake experiment (3 x 3 array).

[1] The Se level in the food is an estimate based on field data from Miller's Reservoir. The tissue level given (13.9 µg Se/g) is for fry netted from Miller's Reservoir that were equivalent to those used in the experiment.

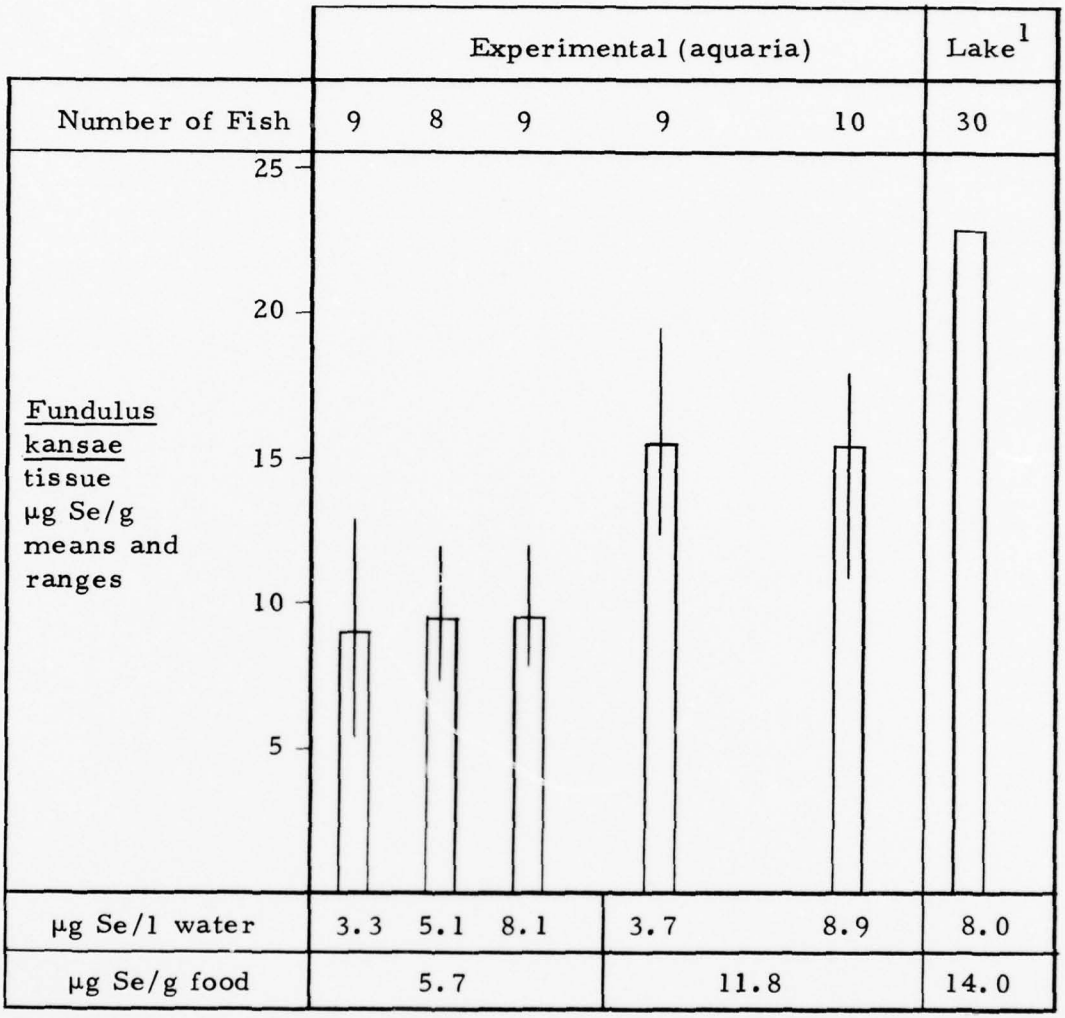

Figure 19. Selenium uptake experiment (2 x 3 array).

[1] The Se level in the food is an estimate based on field data from Twin Buttes Reservoir. The tissue level (22.5 µg Se/g) given is for fry netted from Twin Buttes Reservoir that were equivalent to those used in the experiment.

(treatments) was tested (F-test) and found to be significant at the $\alpha < 0.05$ level. Second, the variability in individual fish weights associated with each of the treatments was tested and found not to be significant. This second test was performed to rule out the possibility that tissue Se level changes might be an artifact caused by some consistent pattern of weight gain difference induced by the experimental arrangement.

Having verified statistically that tissue level variability in both experiments was attributable to food and/or water and not to differential weight gain, multiple comparison methods were used to evaluate the pattern of significant difference between the treatment means. Such comparison is necessary to evaluate whether or not the mean tissue Se level associated with one type of food is significantly different from the mean tissue Se level associated with another type of food regardless of the water type involved (Sokal and Rolf, 1969; Snedecor and Cochran, 1967). Bartlett's test for homogeneity of variance was applied and the variances were found to be sufficiently similar to permit a comparison of treatment means. The Student-Neuman-Keals (SNK) procedure was applied to the parameters of the ANOV table to evaluate the treatment. Tables 13 and 14 portray the SNK results. The means determined not to be significantly different share common underlining. Those not sharing common underlining exhibit a significant difference. In both experiments, it is clear that the highest food Se level (11.8 μg Se/g food) is associated with

significantly higher tissue Se levels than that associated with any lower food level regardless of water type.

A feature of the experimental result that requires interpretation is the fact that most fish "lost" Se from their tissues in the sense that the tissue levels per unit weight were lower at the conclusion of the experiment than they were at the beginning. For purposes of comparison, Figures 16 and 17 include the mean tissue Se levels for fry not subjected to experiment but netted with those that were part of the experiment. Since the fish in both experiments exhibited an average four-fold weight gain during their stay in the aquaria, it appears that the new tissue gained was lower in Se content than the tissue possessed by the fry when netted from their native habitat. This is an expected result because it is probable that most of the food ration types provided to the fish were lower in Se content than their native food (see Figures 18 and 19). If water Se levels were an important contribution to tissue levels via the mechanism of surface absorption, then the tissue Se "loss" phenomena should not have occurred in those cases where fish were kept in water identical to that from which they came.

It should be noted that the waters used in this experiment varied not only in Se content but in other ways as well, such as hardness and dissolved sulfate. Hardness varied over a range from 600 to 5700 mg/l and sulfate varied from 530 to 8600 mg/l, depending on which lake served as the source of the water. As mentioned

earlier, analysis of variance revealed that water variability did not relate to tissue variability. Thus, neither Se level nor the level of other dissolved components such as sulfate or those which contribute to hardness had any effect on tissue Se level.

When the field study results given earlier and the experimental results just discussed are considered together, it is apparent that while organisms such as fish have tissue Se levels which correlate strongly with the levels in the water in which they live, they probably obtain very little of that Se directly from the water. Instead, it is derived from other organisms lower in the food chain, such as algae which do bioconcentrate the element directly from water.

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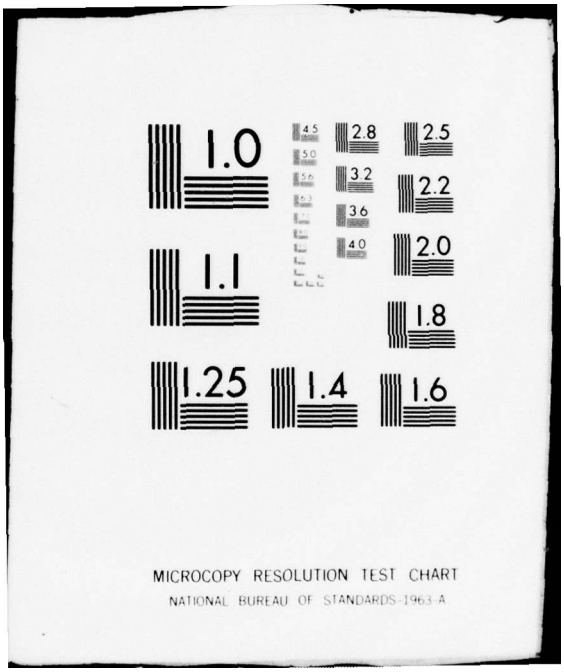
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CHAPTER V

CONCLUSIONS

1. In a survey of 30 aquatic habitats (see Table 3), mean Se levels in the water were found to vary from 0.3 to 15.8 μgSe/1. The levels of Se found in water had no apparent correlation with the level of dissolved sulfate in the water, the hardness of the water, or its conductivity.

2. A detailed study of 17 sites revealed that there is a very strong correlation between the Se levels found in certain organisms and that found in water. Of the invertebrates and fish studied, certain types were shown to bioconcentrate Se in their tissue in a predictable manner. Linear Regression analysis revealed that over the range of water levels encountered, this bioconcentration factor varied between 1300 and 3800, depending on the particular organism involved (see Figure 16). These factors were found to be independent of the levels of dissolved sulfate, the hardness of the water, or its conductivity.

3. Detectable levels of Se in sediment were measured at 14 sites. There was only a weak correlation between these levels and those in water or organisms. However, sediment organic carbon levels were also measured. When the variable μg Se/g sediment

carbon was compared to water and organism Se levels, strong correlations were obtained. The relationships between Se levels in organisms and those in the organic portion of sediments are portrayed as linear regression curves in Figure 17.

4. Experiments with fish indicate that the most important natural source of tissue Se is probably that Se consumed in food rather than that absorbed through exterior surfaces. This confirms earlier work done with radiotracers which suggested that Se enters the food chain principally via uptake by algae and other planktonic forms.

5. The organism tissue Se levels that would be expected to occur in aquatic habitats having water Se levels between 1 and 15 μg/l can be reliably estimated from the curves portrayed in Figure 16. Organism tissue Se levels could also be predicted based on a knowledge of Se and organic carbon levels in sediment using the curves given in Figure 17. For either water or sediment, predictions based on extrapolations beyond the ranges over which measurements were actually made would be less reliable. The accuracy of the prediction could also be reduced if the chemical forms of Se present in the area of concern were different than those encountered in this study (assumed to be primary selenate).

6. As a consequence of the bioconcentration phenomena described above, the tissue Se levels measured in aquatic organisms from the so-called seleniferous habitats studied in this research range up to 60 μg/g when whole body analysis is employed and reach

values exceeding 100 g/g in liver tissue. These levels far exceed those reported for marine habitats or for most inland waters. They are comparable to levels found in terrestrial organisms associated with soils known to have high levels of available Se. The public health implications of this are unclear in view of the uncertainty regarding the toxicity of Se when incorporated into the tissues of aquatic organisms.

7. While providing substantial evidence of bioconcentration of Se, this research provided no evidence that trophic level biomagnification of Se was occurring in the aquatic habitats studied. Moreover, there was no indication that organisms accumulated significantly higher levels of Se with age. However, these conclusions are incidental to the main intent of the study and must be regarded as tentative.

8. In the event that there is concern about the environmental impact of anthropogenic alterations of Se levels in aquatic habitats, biological tissue levels of Se could be used as one measure of the significance of that alteration. Organisms such as those included in Figure 17 could serve as quantitative indicators of the extent to which Se is entering the aquatic food chain. This information, when combined with that obtained from appropriate studies of Se toxicity, would provide a basis for evaluating the potential effects on those organisms (including man) which derive sustenance from the aquatic food chain in question.

LITERATURE CITED

Allaway, W. H., D. P. Moore, J. E. Oldfield, and O. H. Muth. 1966. Movement of physiological levels from soils through plants to animals. J. Nutr. 88: 411-418.

Allaway, W. H. 1973. Selenium in the food chain. The Cornell Veterinarian. 63: 151-170.

Allison, L. E. 1965. Organic carbon. pp. 1367-1378 in Black, C. A., ed. Methods of soil analysis part 2. Chemical and microbiological properties. American Society of Agronomy, Inc., Madison, Wisconsin.

Anderson, M. S., H. W. Lakin, K. C. Beeson, F. F. Smith, and E. Thacker. 1961. Agriculture Handbook No. 200. U. S. Department of Agriculture. Washington, D. C.

Andren, A. W., and David H. Klein. 1975. Selenium in coal-fired steam plant emissions. Environ. Sci. Technol. 9: 856-858.

APHA. 1976. Standard methods for the examination of water and wastewater. 14th ed. American Public Health Association, Washington, D.C.

Associated Press. 1977. Sweitzer Lake fish unsafe to eat. Rocky Mountain News. February 12.

Barnhart, R. A. 1957. Chemical factors affecting the survival of game fish in a western Colorado reservoir. Masters Thesis. Colorado State University, Fort Collins, Colorado.

Baxter, G. T., and J. R. Simon. 1970. Wyoming fishes. Wyoming Game and Fish Department. Cheyenne, Wyoming.

Bertine, K. K., and E. D. Goldberg. 1971. Fossil fuel combustion and the major sedimentary cycle. Science. 173: 233-235.

Bertine, K. K., and E. D. Goldberg. 1972. Trace elements in clams, mussels, and shrimp. Limnol. Oceanogr. 17: 877-884.

Bowen, H. J. M. 1966. Trace elements in biochemistry. Academic Press, London.

Brodie, K. G. 1977. A comparative study--determining arsenic and selenium by AAS. American Laboratory. 9(3): 73-79.

Butler, G. W. and P. J. Peterson. 1967. Uptake and metabolism of inorganic forms of selenium--75 by Spirodela oligorrhiza. Aust. J. Biol. Sci. 20: 77-86.

Byers, H. G. 1935. Selenium in Colorado soils. U. S. Dept. Agr. Tech. Bull. No. 482: 31-32.

Byers, H. G. 1936. Selenium occurrence in certain soils in the United States with a discussion of related topics. Second Report. U. S. Dept. Agr. Tech. Bull. No. 530: 19-24.

Byers, H. G., J. T. Miller, K. T. Williams, and H. W. Lakin. 1938. Selenium occurrence in certain soils in the United States with a discussion of related topics. Third Report. U. S. Dept. Agr. Tech. Bull. No. 601: 1-74.

Chau, Y. K., P. T. S. Wong, B. A. Silverberg, P. L. Luxon, G. A. Bengert. 1976. Methylation of selenium in the aquatic environment. Science 192: 1130-1131.

Chau, Y. K. and P. T. S. Wong. 1977. Selenium in the environment (reply). Science 195: 595.

Committee on Water Quality Criteria. 1972. Water quality criteria. Environmental Protection Agency, Washington, D.C.

Cooper, W. C. and J. R. Glover. 1974. The toxicology of selenium and its compounds. Pp. 654-673 in Zingaro, R. A. and W. C. Cooper, eds., Selenium. Van Nostrad Reinhold Company, New York.

Cooper, W. C., K. G. Bennet and F. C. Croxton. 1974. The history occurrence, and properties of Selenium. Pp. 1-30 in Zingaro, R. A. and W. C. Cooper, eds., Selenium. Von Nostrad Reinhold Company, New York.

Copeland, R. 1970. Selenium: the unknown pollutant. Limnos 3(4): 7-9.

Cowgill, U. M. 1974. The hydrogeochemistry of Linsley Pond, North Branford, Connecticut. Arch. Hydrobiol. Suppl. 45: 1-119.

Crystal, R. G. 1973. Elemental Selenium: structure and proportions. Pp. 13-27 in Klayman, D. L., and W. H. H. Gunther, eds. Organic Selenium compounds: their chemistry and biology. John Wiley Sons, Inc., New York.

Davies, P. H. and W. J. Adrian. 1975. Wet pressure digestion for analysing metals in biological samples of small weight. Outdoor Facts. Fishery Information Leaflet No. 30, Colorado Department of Natural Resources Division of Wildlife, Denver, Colorado.

Delta. 1972. Procedure Manual--Delta Scientific Model 260 Analysis System. Delta Scientific Corporation, Lindenhurst, New York.

Diplock, A. T. 1974. A possible role for trace amounts of Selenium and Vitamin E in two electron-transfer systems of rat-liver microsomes. Pp. 147-160 in Hoekstra, W. G., J. W. Suttie, H. F. Ganther, and W. Mertz, eds. Trace element metabolism in animals--2. University Park Press, Baltimore.

Draper, N. R. and H. Smith. 1966. Applied regression analysis. John Wiley and Sons, Inc., New York, New York.

Environmental Protection Agency. 1975. Preliminary investigation on the effects of the environment of boron, indium, nickel, selenium, tin, vanadium and their compounds. Volume IV. Selenium. National Technical Information Service, United States Dept. of Commerce, Springfield, VA.

Fowler, S. W., and G. Benayoun. 1976. Accumulation and distribution of selenium in mussel and shrimp tissues. Bulletin of Environmental Contamination and Toxicology 16: 339-346.

Fredga, A. Opening speech to the second international symposium on organic selenium and tellurium chemistry including biochemistry. Chemica Scripta 8A.

Frost, D. V. 1972. The two faces of selenium--can selenephobia be cured? Critical Reviews in Toxicology 1: 467-514.

Frost, D. V., and D. Ingvoldstad. 1975. Ecological aspects of selenium and tellurium in human and animal health. Chemica Scripta 8A: 96-107.

Frost, D. V. 1977. Selenium in the environment. Science 195: 594-595.

Ganther, H. E. 1974. Biochemistry of selenium. Pp. 546-614 in Zingaro, R. A. and W. C. Cooper, eds. Selenium. Van Nostrad Reinhold Company, New York.

Ganther, H. E. 1975. Seleleproteins. Chemica Scripta 8A: 79-84.

Ganther, H. E., C. Goudie, M. L. Sunde, M. T. Kopecky, P. Wagner, S. H. Oh, W. G. Hoekstra. 1972. Relation to decreased toxicity of methyl mercury added to diets containing tuna. Science 175: 1122-1124.

Gissel-Nielson, G. and M. Gissel-Nielson. 1973. Ecological effects of selenium application to field crops. Ambio 2: No. 4, 114-117.

Goettl, J. P., and P. H. Davies. 1976. Study of effects of metallic ions on fish and aquaticorganisms, selenium. Pp. 31-34 in Water pollution studies, Colorado Division of Wildlife Job Progress Report, March 1976.

Goulden, P. D. and P. Brooksbank. 1974. Automated atomic absorption determination of arsenic, antimony, and selenium in natural waters. Analytical Chemistry 46: 1431-1436.

Gutenmann, W. H., C. A. Bache, W. D. Youngs, D. J. Lisk. 1976. Selenium in fly ash. Science 191: 966-967.

Hach. 1975. Procedures, chemical lists and glassware for water and wastewater analysis. Hach Chemical Company, Ames, Iowa.

Hamilton, J. W. and O. A. Beath. 1963. Selenium uptake and conversion by certain crop plants. Agron. J. 55: 528-531.

Hashimoto, Y., and J. W. Winchester. 1967. Selenium in the atmosphere. Environ. Sci. Technol. 1: 338-340.

Henn, E. L. 1975. Determination of selenium in water and industrial effluents by flameless atomic absorption. Analytical Chemistry 47: 428-432.

Hill, C. H. 1975. Interrelationships of selenium with other trace elements. Federation of American Societies for Experimental Biology Proceedings. 34: 2096-2100.

Howard, J. H. 1971. Control of geochemical behavior of selenium in natural waters by absorption on hydrous ferric oxides. Pp. 485-495 in Hemphill, D. D., ed., Trace Substances in Environmental Health--V. University of Missouri, Columbia.

Huckabee, J. W. and N. A. Griffith. 1974. Toxicity of mercury and selenium to the eggs of carp (Cyprinus carpio). Trans. Amer. Fish. Soc. 103: 822-825.

Ihnat, M. 1976. Atomic absorption spectrometric determination of selenium with carbon furnace atomization. Analytica Chimica Acta 82: 293-309.

Jervis, R. E., A. Chattopadhyay, E. Csillag and B. Tiefenbach. 1975. Nuclear activation determination of heavy metals in Great Lakes sediments, soils, and vegetation. Pp. 82-94 in Water Quality Parameters (Special Technical Publication 573). American Society for Testing and Materials, Philadelphia, PA.

Johnson, C. M. 1976. Selenium in the environment. Residue Reviews 62: 102-130.

Kharkar, D. P., K. K. Turekian, and K. K. Bertine. 1968. Stream supply of dissolved silver, molybdenum, antimony, selenium, chronium, cobalt, rubidium, and cesium to the ocean. Geochim. Cosmochim. Acta. 32: 285-298.

Kifer, R. R., W. L. Payne, and M. E. Ambrose. 1969. Selenium content of fish meals II. Feedstuffs 41(51): 24.

Koeman, J. H., W. H. M. Peeters, C. H. M. Koudstaal-Hol, P. S. Tjioe and J. J. M. De Goeij. 1973. Mercury-selenium correlations in marine mammals. Nature 245: 385-386.

Koeman, J. H., W. S. M. van de Ven, J. J. M. de Goeij, P. S. Tjioe, and J. L. van Haafteu. 1975. Mercury and selenium in marine mammals and birds. Sci. Total Environ. 3: 279-287.

Lakin, H. W., and D. F. Davidson. 1976. The relation of geochemistry of selenium to its occurrences in soils. Pp. 27-56 in Muth, O. H., ed., Selenium in Biomedicine--First International Symposium, AVI Publishing Company, Westport, Connecticut.

Lakin, H. W. 1972. Selenium accumulation in soils and its absorption by plants and animals. Geological Society of America Bulletin 83: 181-190.

Lakin, H. W. 1973. Selenium in our environment. Pp. 96-111 in Kothny, E. L., ed., Trace elements in the environment--Advances in Chemistry, Series 123. American Chemical Society, Washington, D.C.

Levander, O. A. 1971. Factors that modify the toxicity of selenium. Pp. 57-83 in Mertz, W., and N. W. Cornatzer, eds., Newer Trace Elements in Nutrition, Marcel Dekker, Inc., New York.

Levander, O. A. 1972. Metabolic interrelationships and adaptations in selenium toxicity. N. Y. Acad. Sci. Ann. 192: 193-199.

Luckey, T. D., B. Venugopal and D. Hutcheson. 1975. Heavy metal toxicity, safety and hormology. Pp. 5-71 in Coulston, F., ed., Environmental Quality and Safety. Academic Press, New York.

Lunde, G. 1970a. Analysis of trace elements in seaweed. J. Sci. Food Ag. 21: 416-418.

Lunde, G. 1970b. Analysis of arsenic and selenium in marine raw materials. J. Sci. Food Ag. 21: 242-247.

Mackay, N. J., M. N. Kazacos, R. J. Williams and M. I. Leedow. 1975. Selenium and heavy metals in black marlin. Mar. Pollut. Bull. 6: 57-61.

Martin, J. L. 1973. Selenium assimilation in animals. Pp. 663-691 in Klayman, D. L., and W. H. H. Gunther, eds., Organic Selenium Compounds: Their Chemistry and Biology. Wiley, New York.

Merrit, W. F. 1975. Variation in trace element concentrations along the length of the Ottawa River. Can. J. Earth Sci. 12: 850-857.

Mitchell, Ralph. 1974. Introduction to environmental microbiology. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

Moore, T. D. 1976. A survey for selenium in waters and fish tissues from selected lakes and springs in Wyoming. Wyoming Game and Fish Research Laboratory, Laramie, Wyoming.

Morris, V. C., and O. A. Levander. 1970. Selenium content of foods. J. Nutr. 100: 1383-1388.

Moxon, A. L. and O. E. Olsen. 1974. Selenium in agriculture. Pp. 675-707 in Zingaro, R. A. and W. C. Cooper, eds., Selenium. Van Nostrad Reinhold Company, New York.

NBS. 1971. National Bureau of Standards certificate of analysis. Standard reference material 1571. Orchard leaves. U. S. Department of Commerce, Washington, D. C.

NBS. 1972. National Bureau of Standards certificate of analysis. Standard reference material 1577. Bovine liver. U. S. Department of Commerce, Washington, D. C.

Nie, H. H., C. H. Hall, J. G. Jenkins, K. Steinbrenner, D. H. Bent. 1975. Statistical package for the social science. McGraw Hill Book Company.

Niimi, A. J., and Q. N. LaHam. 1975. Selenium toxicity on the early life stages of zebrafish (Brachydanio rerio). J. Fish Res. Can. 32: 803-806.

Niimi, A. J., and Q. N. LaHam. 1976. Relative toxicity of organic and inorganic compounds of selenium to newly hatched zebra fish (Brachydanio rerio). Can. J. Zool. 54: 501-509.

Oldfield, James E. 1974. Selenium. Pp. 57-63 in Geochemistry and the Environment. National Academy of Science, Washington, D. C.

Olsen, O. E. 1967. Soil, plant, animal cycling of excessive levels of selenium. Pp. 297-312 in Muth, O. H., ed., Selenium in Biomedicine--First International Symposium. AVI Publishing Company, Westport, Connecticut.

Pakkala, I. S., W. H. Gutenmann, D. J. Lisk, G. E. Burdick, and E. J. Harris. 1972. A survey of the selenium content of fish from 49 New York State waters. Pesticides Monitoring Journal 6: 107-114.

Parizek, J., I. Ostadalova, T. Kaluskova, A. Babicky, and J. Benes. 1971. Pp. 85-122 in Mertz, W., and W. E. Cornatzer, eds., Newer Trace Elements in Nutrition. Marcel Dekker, Inc., New York.

Patrick, R., T. Bott, and R. Larson. 1975. The role of trace elements in management of nuisance growths. EPA-660/2-75-008. U. S. Environmental Protection Agency, Corvallis, Oregon.

Pennak, Robert W. 1953. Freshwater invertebrates of the United States. The Ronald Press Company, New York, N. Y.

Perkin Elmer. 1976. Analytical method: for atomic absorption spectrophotometry. Perkin Elmer Instrument Division, Norwalk, Connecticut.

Pierre, F. D., T. C. Lamoreaux, H. R. Brown and R. S. Fraser. 1976. An automated technique for the submicrogram determination of selenium and arsenic in surface waters by atomic absorption spectroscopy. Applied Spectroscopy 30: 38-42.

Pratt, D. R., J. S. Bradshaw, and B. West. 1972. Arsenic and selenium analysis in fish. Utah Academy Proceedings 49: 23-26.

Rosenfeld, I., and O. A. Beath. 1964. Selenium, geobotany, biochemistry, toxicity, and nutrition. Academic Press, New York.

Rossi, L. C., G. F. Clemente, and G. Santaroni. 1976. Mercury and selenium distribution in a defined area and in its population. Arch. Environ. Health 31: 160-165.

Sandholm, M., H. E. Oksanen, L. Pesonen. 1973. Uptake of selenium by aquatic organisms. Limnology and Oceanography 18: 496-499.

Schroeder, H. A. 1974. The poisons around us. Indiana University Press, Bloomington.

Schwarz, K., and K. D. Pathak. 1975. The biological essentiality of selenium, and the development of biologically active organoselenium compounds of minimum toxicity. Chemica Scripta 8A: 85-95.

Scott, M. L. 1973. Nutritional importance of selenium. Pp. 629-661 in Klayman, D. L., and W. H. H. Gunther, eds., Organic Selenium Compounds: Their Chemistry and Biology. John Wiley & Sons, Inc., New York.

Scott, R. C., and P. T. Voegeli. 1961. Radiochemical analyses of ground and surface water in Colorado, 1954-1961. Colorado Water Conservation Board Basic Data Report No. 7: 14-27.

Shapiro, J. R. 1973. Selenium and human biology. Pp. 693-726 in Klayman, D. L., and W. H. H. Gunther, eds., Organic Selenium Compounds: Their Chemistry and Biology. John Wiley & Sons, Inc., New York.

Shrift, A. 1973. Metabolism of selenium by plants and micro organisms. Pp. 763-814 in Klayman, D. L., and W. H. H. Gunther, eds., Organic Selenium Compounds: Their Chemistry and Biology. Wiley, New York.

Sielicki, M., and J. C. Burnham. 1973. The effect of selenite on the physiological and morphological properties of the blue-green alga Phormidium luridum var. olivacea. J. Phycol. 9: 509-514.

Snedecor, G. W., and W. G. Cochran. 1967. Statistical methods, 6th ed. The Iowa State University Press, Ames, Iowa.

Sokal, R. R., and F. J. Rohlf. 1969. Biometry. W. H. Freeman and Company, San Francisco.

Stadtman, T. C. 1974. Selenium biochemistry. Science 183: 915-922.

Subcommittee on Selenium. 1976. Medical and biologic effects of environmental pollutants, selenium. National Academy of Sciences, Washington, D. C.

Underwood, E. J. 1971. Trace elements in human and animal nutrition, 3rd ed., Academic Press, New York.

USGS. 1955. Geological map of Wyoming. U. S. Geological Survey, Washington, D. C.

USGS. 1967. State of Wyoming, base map with highways and contours. U. S. Geological Survey, Washington, D. C.

USGS. 1973. State of Colorado base map with highways and contours. U. S. Geological Survey, Washington, D. C.

USGS. 1975. Geologic map of Colorado. Colorado Geological Survey, Denver, Colorado.

Usinger, Robert L., ed. 1956. Aquatic insects of California with keys to North American Genera and California species. University of California Press, Santa Barbara, California.

Uthe, J. F., and E. G. Bligh. 1971. Preliminary survey of heavy metal contamination of Canadian fresh water fish. J. Fish Res. Board Can. 28: 786-788.

Weir, P. A., and C. H. Hine. 1970. Effects of various metals on behavior of conditioned goldfish. Arch. Environ. Health 20: 45-51.

Wentz, D. A. 1974. Effect of mine drainage on the quality of streams in Colorado, 1971-72. Colorado Water Resources Circular No. 21. Colorado Water Conservation Board, Denver, Colorado.

Wetzel, R. G. 1975. Limnology. W. B. Saunders Company, Philadelphia, Pennsylvania.

Wiersma, J. H., and G. F. Lee. 1971. Selenium in lake sediments. Analytical procedure and preliminary results. Environ. Sci. Technol. 5: 1203-1206.

Williams, K. T., and H. G. Byers. 1935. Occurrence of selenium in the Colorado River and some of its tributaries. Industrial and Engineering Chemistry 7: 431-432.

Woodwell, G. M. 1967. Toxic substances and ecological cycles. Scientific American 216(3): 24-31.

APPENDICES

APPENDIX 1

The Chemistry of Selenium and Its Importance in Metabolism

Selenium (Se) is one of about ten trace elements that are known to be essential for higher animals (Underwood, 1971). Se is also one of the many elements regarded as a potentially toxic environmental pollutant (Oldfield, 1974; Subcommittee on selenium, 1976). The knowledge that this element had both beneficial and harmful aspects began accumulating in the 1930's as a result of purely practical problems encountered in the raising of livestock and poultry (Allaway, 1973). Investigations revealed that some producers in the western plains were suffering losses because of too much Se in the forage consumed by their cattle. Producers elsewhere were suffering losses because of too little Se in the feed of their poultry. Now it is known that there is a lower and an upper limit to the amount of Se that should be in feed. For the chemical forms of Se that occur in cultivated forage, this range is currently estimated to be appropriate if it is between 0.04 and 4.0 micrograms per gram (parts per million) (Oldfield, 1974; Lakin, 1973). To prevent the conditions attributed to too much Se, feeding on seleniferous forage (wild or cultivated) must be curtailed. To prevent deficiency conditions, various measures have been tried. Compounds of Se can be administered in several forms including additions to the food or to the soil on which the food is grown

(Allaway et al., 1966). The mixing of feeds from various sources also helps to alleviate both the excess and deficiency problem (Allaway, 1973).

Understandably, in the present era of acute environmental awareness and concern for food adulteration, the advisability of adding substances such as Se compounds to fertilizer or to feed was open to question (Shroeder, 1974). This was especially true of Se because tests initiated in the 1940's with experimental animals led some investigators to label the element as a carcinogen. Since other researchers were unable to confirm these findings, a vigorous controversy arose. Those who "needed" Se (animal husbandry interests) and those "opposed" to it (those concerned about toxicity) were joined by those who had it to "sell" (Frost, 1972). These were the producers of copper and other metals who were seeking markets for the Se produced as a by-product of copper, zinc, nickel, silver, and uranium refining (Crystal, 1973; Cooper et al., 1974). While the controversy continues (Frost, 1977; Chau and Wong, 1977), its intensity has subsided because better information has come to light regarding the behavior of the various forms of Se in living systems (Subcommittee on selenium, 1976).

The chemical behavior of Se is most similar to that of sulfur. The outer electron configuration resembles that of sulfur and both are Group VIA elements on the periodic Table (Luckey et al., 1975). Selenium's atomic number is 34. This is only one greater than arsenic,

another element with which it shares some common characteristics (Crystal, 1973) and one less than bromine. While not naturally occurring, radioactive Se isotopes have been prepared (Rosenfeld and Beath, 1964). One of these, ^{75}Se, has been incorporated into many molecular combinations and has proved very useful as a research tool partly because it behaves as a sulfur analog in some biochemical reactions (Shapiro, 1973). Like sulfur, Se has -2, 0, +4, and +6 oxidation states (Rosenfeld and Beath, 1964). Examples of the -2 state (most reduced) are volatile compounds such as hydrogen selenide (H_2Se) and dimethyl selenide $\left[(CH_3)_2Se\right]$, and the selenium incorporated into amino acids such as selenomethionine. Elemental Se^0 assumes different isomeric forms, some of which resemble sulfur in appearance as well as one which is metallic in appearance. The +4 state can take the form of selenium dioxide (SeO_2) or the selenite ion (SeO_3^{-2}). The +6 state (most oxidized) can exist as hydrated selenic acid ($H_2SeO_4 \cdot H_2O$) or the selenate ion (SeO_4^{-2}). The ions form crystalline salts with metals as do the corresponding sulfites and sulfate ions. The oxidation-reduction potentials for Se differ from sulfur, however, in that for a given valence state, Se is oxidized with more difficulty than its sulfur counterpart (Lakin, 1973). Another consistent difference is that Se compounds have higher melting and boiling points than their sulfur analogs. The fact that Se and sulfur are similar but not identical in properties, has important consequences for their respective biochemical behaviors. For example, because sulfur

is a component of the amino acid methionine, there exists a counterpart called selenomethionine in which Se atoms replace sulfur atoms. For some enzyme systems which handle methionine, the substitution seems to make little difference, but the two amino acids are by no means completely alike in their metabolic and physical properties (Shrift, 1973). Selenocysteine is another example of a Se-for-sulfur-substituted molecule.

Biochemical explanations for the positive role that Se plays in metabolic function are being actively sought. It has been characterized as the most potent micronutrient known (Schwarz and Pathak, 1975). It has been determined that Se atoms are an integral part of certain enzyme molecules. The precise stoichiometric amounts of Se are known for the glutathione peroxidase found in mammalian erythrocytes and bacteria. Another Se-containing molecule is a component of the glycine reductase complex of Clostridium stiklandii. There is also evidence of a role for Se in microbial formic dehydrogenase and in the electron transport chain of rat liver mitochondria (Ganther, 1975; Diplock, 1974). Thus, at least part of the explanation for Se essentiality seems to be its presence in enzyme molecules which are necessary for normal metabolic function. Other benefits derived from Se may be related to its protective interactions with other elements (Levander, 1971). Some investigators (Ganther et al., 1972) have suggested that the "tuna fish scare" involving what was believed to be excessive mercury levels in tuna fish being caught for human consumption was

unwarranted because Se was also present in the tuna. Both Se and mercury are present in sea water and both are accumulated in the tuna tissues. This co-occurrence of two elements may serve to protect the tuna and the consumer of the tuna from the effects mercury would have if it were present alone. These mutually protective effects have also been suggested for marine mammals and birds (Mackay et al., 1975; Koeman et al., 1975). The protective effect of one element against the effects of others is a well documented phenomenon (Frost and Ingvoldstad, 1975). Se interactions with cadmium and arsenic have also been studied in this regard (Parizek et al., 1971; Hill, 1975). Other suspected effects ascribed to Se include reducing the incidence of certain types of cancer and vascular disease in man (Subcommittee on selenium, 1976).

As for the harmful effects of a dietary excess of Se, earlier claims about its carcinogenic properties have proven unfounded, but there is no doubt that various forms of the element can produce both acute and chronic toxicity. Se has been described as the most toxic, weight for weight, of all the essential elements (Frost and Ingvolstad, 1975). To explain this, it has been suggested that selenium's toxicity mechanism may resemble those of arsenic and cadmium with the vascular system being one of the primary sites of action (Subcommittee on selenium, 1976). One hypothesis is that Se replaces sulfur in thiol groups in dehydrogenase enzyme systems and thereby acts to inhibit cellular respiration (Luckey et al., 1975). It should be noted

that the narrow range of tolerance between deficiency and toxicity of Se exhibited by animals is not observed in plants. With the possible exception of the small group of Se accumulating plants found only in seleniferous regions, plants have not been shown to require Se for growth. However, high soil levels of Se can inhibit plant growth. The degree of inhibition depends on soil levels of sulfate, which acts as a competitive antagonist (Shrift, 1973). The question as to how plants take up various forms of Se and how it is incorporated into plant tissue is a subject of active research. It may be that the similarity between Se compounds and their sulfur counterparts allows both elements to share some common metabolic pathways (Shrift, 1973).

In view of selenium's obvious importance to animal health, Se supplements have been approved for use in animal nutrition. This was done once the questions concerning carcinogenic properties and environmental impact effects had been settled to the satisfaction of the regulating agencies involved. In New Zealand, where the deficiency problem is especially acute, feed supplementing began in 1959 (Frost, 1972). Supplementing of poultry (except laying hens) and swine feed was approved in the United States in 1973 (Subcommittee on selenium, 1976). Fertilizer supplementing to increase Se levels in forage has not been approved because of the prevailing opinion that this approach is both inefficient and hazardous (Allaway et al., 1966).

One of the factors which had to be considered before approving dietary Se supplements was the absorption and excretion dynamics

exhibited by various chemical forms of the element (Johnson, 1976). Selenate, selenite, and naturally occurring organic forms of Se are all readily taken up in the small intestine by monogastric animals and with less efficiency by ruminants. The form of Se approved for dietary supplements is selenite or selenate. When Se is administered at the appropriate levels (0.2 μg/g) in either of these forms, deficiency in the tissues is remedied with the excess being excreted. Excretion is principally via the urine, with trimethyl selenomium ion $\left[(CH_3)_3Se^+\right]$ being one of the major forms. The feces and the lungs have a lesser role. In cases where there is excessive dietary selenate or selenite, the lungs can play a greater role by increased excretion of volatile dimethyl selenide $\left[(CH_3)_2Se\right]$ (Martin, 1973). Organic selenium forms do not necessarily behave in this manner. Cultivated plant protein containing amino acids which incorporate Se such as seleno-methionine, or which have Se bound to them in some fashion, are not metabolized in such a way as to ensure that the excess Se will be excreted. Tissue accumulation can occur and chronic Se toxicity symptoms can appear (Subcommittee on Selenium, 1976).

APPENDIX 2

Microbial Transformations of Selenium Compounds

Selenium (Se) transformation by micro-organisms is a process that must be included when considering the dynamics which influence Se levels in soil and water. Reduction of Se compounds is apparently a relatively more widespread phenomenon than is oxidation (Shrift, 1973).

There is little doubt that reduction of Se does occur in both soil and sediment systems and that several bacteria and fungal species are involved (Chau et al., 1976; Ganther, 1974). Selenite (SeO_4^{-2}) and selenite (SeO_3^{-2}) are both reducible to either elemental Se, volatile methylated forms, or incorporated protein components such as selenomethionine. The magnitude of this activity in nature is not known and the importance of it in Se cycling is only conjectural. Unlike the methylated forms of mercury which have been so widely publicized as dangerous pollutants, methylated Se compounds are not considered to be very toxic although a toxic synergistic interaction with mercury salts has been described (Subcommittee on selenium, 1976).

The role of biological Se oxidation in nature is even more poorly understood than is reduction. Microbial oxidation of Se such as the conversion of elemental Se or selenite to selenate has been observed

only under laboratory conditions and for a very limited number of organisms (Shrift, 1973).

Given the apparent nature of microbial Se transformation as reported in the literature, the effect would seem to be either to disperse Se as a volatile organic compound, to incorporate it as part of microbial protein, or to convert to the relatively inert elemental form.

APPENDIX 3

Selenium Toxicity to Aquatic Organisms

The potential for possible harm to aquatic organisms by selenium (Se) has been investigated and acknowledged. A recommendation (Committee on Water Quality Criteria, 1972) was made for allowable Se contamination in the marine environment. Bioassay studies were cited wherein 48-hour exposure to sodium selenite (Na_2SeO_3) at levels of 2500 μg/l produced threshold toxic effects in Daphnia. Further citing that bioconcentration of Se in the food chain leading to fish was believed to be a possibility, the maximum allowable level was set at 10 μg/l.

Detrimental Se level determinations using the behavioral responses of goldfish have also been performed (Weir and Hine, 1970). Selenium dioxide (SeO_2) which forms selenite (SeO_3^{-2}) when dissolved was used in these experiments. The lowest level which interferred with conditioned responses in the fish was reported to be 250 μg/l. Lethal concentration levels were also measured. Fifty percent mortality in 48 hours occurred at a level of 12000 μg/l.

Se effects on embryonic fish have also been investigated (Niimi and LaHam, 1975). Prior to hatching, Zebrafish (Brachydanio rerio) were unaffected by Na_2SeO_3 in the 500-10,000 μg/l range. After hatching, levels above 3000 μg/l proved 90 percent fatal in 10 days. In a later study, the same investigators (Niimi and LaHam, 1976)

compared the effects of various selenite (SeO_3^{-2}) and selenate (SeO_4^{-2}) salts on Zebrafish and found selenite to be less toxic than selenite. To produce 50 percent mortality in 96 hours, 82,000 μg selenate was required compared to 23,000 μg selenite/l. The effect of selenium dioxide on carp (Cyprinus carpio) eggs was studied by Huckabee and Griffith (1974). No significant effects at levels up to 5000 μg/l were observed.

Recent long-term (one year) work with rainbow trout indicate that selenite levels as low as 40 μg/l produce abnormal morphological development (Goettl and Davies, 1976). These investigators also performed 16-day acute toxicity tests with selenite and found that 5000 μg/l produced 50 percent mortality.

Algal responses to Se have also been studied. The effect of sodium selenite on the behvaior of culture of the blue-green alga Phormidium ivridum was investigated by Sielicki and Burnham (1973). Concentrations of less than 150,000 μg/l were not inhibitory to photosynthesis. Above this level there was a progressive increase in inhibition as well as detrimental changes in cell structure. The authors also cited other studies with blue-green algae in which similar results were obtained.

It should be noted that nearly all the studies described above involved biological responses to selenite. This is not likely to be prevalent form in water and the results obtained with selenite may not be representative of the way Se effects would occur in aquatic

habitats. Moreover, the levels of Se used in most of the studies would not occur in aquatic habitats.

APPENDIX 4

Selenium Levels in Terrestrial Organisms

The most studied aspect of biological selenium (Se) levels has been of those organisms in the food chain which culminates in domesticated animals and man. Both vegetation and meat levels tend to correlate with the Se availability in soils at their place of origin (Allaway, 1973). Animal feed concentrations vary from 0.01 μg/g (Pacific Northwest or Atlantic Coast) to 5 μg/g (Central United States). Wheat varies from 1 to 15 μg/g depending on origin. Cabbage from a seleniferous farm in South Dakota had 100 μg/g. Cabbage from a seleniferous area in Ireland had 409 μg/g. For meat, levels in adequately nourished animals range from 0.1 to 0.5 μg/g (Ganther, 1974). Animals with levels in the 0.01-0.05 μg/g range are considered to be deficient in Se.

In healthy animals, the internal distribution exhibits the following pattern from highest to lowest tissue level: kidney, liver, pancreas, lungs, heart, muscle (Scott, 1973). Liver values have been extensively reported and fall into the 1 to 10 μg/g range although the value can be as low as 0.04 μg/g in low Se areas and as high as 60 μg/g in animals from seleniferous regions (Johnson, 1976).

Although not well studied, Se levels in man seem not to differ from those found in his food animals. Wide variations are not very prevalent in man nor are deficiency and excess disease syndromes.

This is attributed to the wide variety of food items and food sources available to most persons (Shapiro, 1973; Rossi et al., 1976).

Another major area of inquiry concerning Se levels in organisms has involved the "accumulator plants" that are found in seleniferous habitats. They are also called "indicators" or "converters" by some authors because their presence is an indication of high soil Se levels and they are thought to convert Se from one form to another. Certain species from genera such as Astragulus and Stannleya are known as primary Se accumulators and are found only on highly seleniferous soils. When consumed by livestock, these plants are responsible for the acute disease syndrome known locally in the western plains as blind staggers (Rosenfeld and Beath, 1964). These plants accumulate Se to levels typically in the 1000 to 10,000 μg/g range. The form of selenium accumulated is unique in that it is a water soluble non-protein amino acid form. Examples of these amino acids are Se-methyl selenocysteine and selenocystathionine. Another feature of the accumulators is that some possess poisonous alkaloids which may be even more responsible for the syndrome than is Se (Lakin, 1973). Insects have been found feeding on seeds and other plant parts belonging to primary accumulators. Larvae exhibited levels up to 10 μg/g and adults as high as 67 μg/g (Byers et al., 1938).

Another class of plants called secondary accumulators exhibit Se values in the 0 to 100 μg/g range. Plants in this category such as wild aster (Aster) and Saltbush (Atriplex) are not confined to

seleniferous regions and in addition to protein bound forms, accumulate soluble inorganic forms of Se such as selenate (SeO_4^{-2}).

When cultivated crops and native grasses are grown in regions of high Se availability, they are said to be capable of accumulating up to 30 μg/g Se in primarily protein-bound form (Rosenfeld and Beath, 1964).

Recent work involving even higher levels was done with clover (Melilotus alba) in a man-made seleniferous area. The plants were growing on landfill consisting of flyash precipitated from four coal-burning power plants in New York State (Gutenmann et al., 1976). The highest levels found were 69 μg/g in plants growing in flyash containing 21.0 μg/g Se. The upper part of the plant contained 200 μg/g.

Another report described the effects of selenite (SeO_3^{-2}) additions to soil on Danish farms. A mean value of 7.5 μg/g in earthworms (Lumbricus terrestris) was measured versus 2.2 μg/g in controls from areas not supplemental with selenite. The range observed for centipedes was from 4.0 μg/g in those exposed to soil with added selenite versus 1.5 μg/g in controls. For woodlice and crane fly larvae the range was 1.5 μg/g in those exposed to 1.0 μg/g in controls (Gissel-Nielson and Gissel Nielson, 1973).

APPENDIX 5

Water Analysis with the Heated Graphite Atomizer (Carbon Rod)

In theory, analysis of water levels of selenium (Se) is carried out in the carbon rod atomizer in a manner that is similar to the method used to analyze prepared biological materials (Brodie, 1977). In practice, however, water analysis is subject to severe interferences not encountered with biological samples.

Trial analyses revealed that high levels of sulfate ion (SO_4^{-2}) can cause up to 60 percent suppression of the Se absorbance reading. This must be compensated for by the method of standard additions where various known amounts of Se are added to replicate samples (Perkin-Elmer, 1976).

Excessive cation levels are also a problem. Elements such as magnesium (Mg) cause a highly exaggerated absorbance reading (as much as five-fold) due to non-atomic absorption probably caused by spattering of the charred sample during atomization (Henn, 1975). This interfering absorbance was beyond the capability of the instrument's background corrector to remedy. The phenomenon affected several wave-lengths in addition to the 195.4 nm found most suitable for Se analysis. According to the information supplied by the manufacturer (Perkin-Elmer), the only compensation for this kind of error is to remove the cations from the sample prior to analysis. A

technique for doing this (Henn, 1975) using cation exchange resin followed by Molybdenum (Mb) additions was tried. This procedure is time consuming but was judged effective in dealing with the interference.

Another disadvantage of the carbon rod analysis technique is the recommended digestion procedure (APHA, 1976). This involves repeated refluxing and evaporation to dryness with nitric acid (NHO_3) and hydrogen peroxide (H_2O_2) with the residue being taken up in concentrated hydrochloric acid (HCL).

This investigator chose not to employ carbon rod atomic absorption analysis because many of the water samples in this study were very heavy in dissolved solids including sulfate and magnesium. To have employed the carbon rod approach would have required a complicated digestion, pretreatment with cation exchange resin, and an analytical procedure made more time-consuming by the requirement for standard additions. Moreover, the increased number of steps and frequent handling of the sample considerably increases the chances of Se loss or contamination.

The principal reason why biological sample analysis is not subject to the interference described above is probably the fact that the digested sample is diluted (at least 20-fold in this research) sufficiently to reduce the problem to negligible levels.

APPENDIX 6

Selenium Levels in Water, Sediment, and Organisms[1]
(all units are μg Se/g dry weight unless otherwise indicated)

Study Site[2]	Park Creek				Meeboer				Diamond			
Water (μg Se/l)	3	0.2-0.3	0.3	0.1	3	0.2-0.4	0.3	0.1	3	0.3-0.4	0.3	0.1
Hyalella azteca					4	1.8-2.8	2.3	0.5	4	1.4-1.7	1.6	0.2
Astacidae	4	1.1-1.3	1.2	0.1								
Cenocorixa Sp.	2	2.1-2.4	2.3	0.2	4	2.1-6.1	4.2	1.9	4	2.2-2.5	2.3	0.1
Coenagrionidae					4	2.6-4.1	3.1	0.8	2	1.7-1.8	1.8	0.1
Chironomus Sp.					1		2.5					
Fish Fry	2	0.3-0.5	0.4	0.1					2	1.6-1.7	1.6	0.1
Fundulus kansae												
Pimephales promelas	3	2.0-2.2	2.1	0.1					1		2.1	
Etheostoma exile												
Plankton					2	3.0-3.8	3.4	0.6				
Filamentous Algae												
Chara Sp.					Below detection				Below detection			
Sediment	Below detection				2	0.2-0.4	0.3	0.1	Below detection			
Organic Sediment (μg Se/g carbon)	Below detection				2	6-9-11.4	9.4	3.2	Below detection			

Study Site	Gelatt				Hasty				#64			
Water (μg Se/l)	3	0.4-1.5	0.8	0.6	3	0.6-0.8	0.7	0.1	3	0.4-2.8	1.7	1.2
Hyalella azteca	4	2.1-3.1	2.9	0.5					4	3.2-4.3	4.0	0.5
Astacidae									2	5.4-5.4	5.4	
Cenocorixa Sp.	2	4.1-4.4	4.2	0.2	4	3.0-7.1	4.2	2.0	2	5.3-6.4	5.9	0.8
Coenagrionidae	4	3.7-5.2	4.4	0.7	2	10.9-11.2	11.1	0.2	3	7.0-7.8	7.7	0.2
Chironomus Sp.									2	8.1-9.1	9.0	0.2
Fish Fry	2	1.7-2.5	2.1	0.6	2	4.3-4.8	4.6	0.3	2	7.4-8.0	7.7	0.4
Fundulus kansae					2	4.3-4.8	4.6	0.4				
Pimephales promelas												
Etheostoma exile	2	1.7-2.5	2.1	0.6								
Plankton	2	3.3-3.4	3.3	0.1	2	7.7-8.1	7.9	0.3	2	4.3-4.4	4.4	0.1
Filamentous Algae	2	0.4-0.5	0.5	0.1								
Chara Sp.												
Sediment	2	2.6-3.0	2.8	0.3	Below detection				2	3.2-3.5	3.3	0.2
Organic Sediment (μg Se/g carbon)	2	41.3-57.7	49.5	11.6	Below detection				2	106.-113.	110.	4.4

Study Site	Timber				Mac Mesa				Cobb			
Water (μg Se/l)	3	1.6-2.7	2.2	0.7	3	1.9-2.9	2.2	0.6	3	2.9-6.4	3.8	2.3
Hyalella azteca	2	4.2-4.4	4.3	0.1								
Astacidae	3	8.0-11.7	10.1	1.9	2	3.9-5.5	4.7	1.1				
Cenocorixa Sp.									4	7.2-9.3	8.4	1.0
Coenagrionidae	3	23.6-36.4	28.4	7.0	4	10.0-11.9	11.2	0.9				
Chironomus Sp.	4	14.8-16.7	15.3	0.9								
Fish Fry												
Fundulus kansae												
Pimephales promelas												
Etheostoma exile												
Plankton					2	70.8.3	7.7	0.9	2	7.4-8.0		7.7
Filamentous Algae	2	16.5-17.0	16.8	0.4					2	7.4-7.7	7.6	0.2
Chara Sp.	2	0.4-0.2	0.3	0.1								
Sediment	2	33.3-57.1	46.	16.9	2	1.4-2.1	1.8	0.5	2	3/0-4/8	4/3	0.6
Organic Sediment (μg Se/g carbon)					2	66.7-110.	88.	31.0	2	169.-180.	176	8.3

Study Site	Highline				East Allen				Miller's			
Water (μg Se/l)	3	3.9-4.5	4.2	0.3	3	3.8-5.3	4.8	0.8	3	5.3-7.4	6.0	1.2
Hyalella azteca	2	4.2-4.4	4.3	0.1	4	10.8-12.3	11.4	1.6	2	7.2-7.9	7.6	0.5
Astacidae	3	8.0-11.7	10.1	1.9					2	10-5.12.1	11.3	1.1
Cenocorixa Sp.					2	10.9-11.0	11.0	0.1	4	8.9-10.1	9.9	0.7
Coenagrionidae	3	23.6-36.4	28.4	7.0	4	18.0-20.1	18.7	0.9	4	14.8-17.0	15.8	0.9
Chironomus Sp.	4	14.8-16.7	15.3	0.9					4	17.3-20.6	18.8	1.7
Fish Fry									2	13.9-14.2	14.1	0.2
Fundulus kansae												
Pimephales promelas									5	8.7-16.6	11.0	3.4
Etheostoma exile					1		36.3		3	21.7-24.5	23.0	1.4
Plankton					2	10.1-11.8	11.0	1.2				
Filamentous Algae					2	2.9-3.0	3.0	0.1	2	4.4-4.7	4.6	0.2
Chara Sp.	2	16.5-17.0	16.8	0.4								
Sediment	2	0.8-1.6	1.2	0.6	2	4.0-4.1	4.1	0.1	2	4.4-4.4	4.4	
Organic Sediment (μg Se/g carbon)	2	53.3-100.	77.	33.0	2	114.-114.	114.		2	258.-275.	267.	11.4

Study Site	Twin Buttes				Duck				Sweitzer			
Water (μg Se/l)	3	7.5-7.8	7.6	0.2	3	7.0-12.0	9.1	2.6	3	8.7-12.0	9.4	2.3
Hyalella azteca	4	9.6-13.1	11.3	1.6								
Astacidae												
Cenocorixa Sp.	4	13.4-17.0	15.5	1.5	4	12.6-16.5	14.4	1.8				
Coenagrionidae	4	11.9-25.1	18.4	6.5	2	21.9-21.9	21.9		4	30.3-62.8	45.1	16.1
Chironomus Sp.	2	31.1-37.3	34.2	4.3	2	22.0-24.3	23.1	1.6	2	26.7-26.7	26.7	
Fish Fry	2	21.2-22.5	21.9	0.9					2	21.8-29.5	25.7	5.4
Fundulus kansae	2	21.6-24.5	23.1	2.0					4	21.8-53.3	31.9	14.6
Pimephales promelas	4	31.1-37.3	34.5	3.0					2	76.8-81.1	79.0	3.0
Etheostoma exile	1		41.9									
Plankton	2	15.2-15.5	15.4	0.2	2	17.8-23.8	20.8	4.2	2	41.1-43.8	42.5	1.9
Filamentous Algae	2	7.3-8.2	7.8	0.6					2	6.5-6.5	6.5	
Chara Sp.									4	7.1-14.6	14.2	0.6
Sediment	2	10.5-11.0	10.8	0.4	2	2.0-2.2	2.1	0.1	2	7.1-5.8	6.5	0.9
Organic Sediment (μg Se/g carbon)	2	339.-355.	347.	11.4	2	143.-169.	156.	18.6	2	527.-645.	586.	83.6

Study Site	Desert				Pond #9			
Water (μg Se/l)	2	7.0-18.0	12.5		3	11.5-22.0	15.8	7.8
Hyalella azteca	2	18.0-18.8	18.4	0.6	4	19.4-26.5	22.5	3.3
Astacidae	5	12.5-34.0	23.3	9.4	2	36.0-37.5	36.8	1.1
Cenocorixa Sp.	4	21.6-37.4	29.4	6.8				
Coenagrionidae	1		55.0		3	46.5-62.8	53.3	8.5
Chironomus Sp.	4	44.7-70.6	58.2	12.8	2	59.1-59.5	59.3	0.3
Fish Fry	2	58.1-63.1	60.6	3.5	2	66.5-67.9	67.2	1.0
Fundulus kansae					6	47.6-65.4	57.4	6.2
Pimephales promelas								
Etheostoma exile								
Plankton	2	31.0-31.5	31.3	0.4				
Filamentous Algae					2	20.7-21.0	20.9	0.2
Chara Sp.	2	8.7-8.8	8.8	0.1	2	12.9-14.5	13.7	1.1
Sediment	2	15.2-15.5	15.4	0.2	2	47.1-47.4	47.3	0.2
Organic Sediment (μg Se/g carbon)	2	844.-969.	902.	87.8	2	1208.-1215.	1212.	5.4

[1] Each entry is arranged as follows: Number of Determinations, Range, Mean, Standard Deviation.

[2] Locations are given in Table 3.